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UNIVERSITY OF ALBERTA

BEHAVIOURAL AND NEUROCHEMICAL STUDIES OF TRYPTAMINE IN THE RAT

by

DARRELL DAVID MOUSSEAU

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

IN

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FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

AND FACULTY OF MEDICINE (PSYCHIATRY)

EDMONTON, ALBERTA

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1 ABSTRACT

Manipulations of incubation medium conditions demonstrated that [^{3}H]-tryptamine binding in the rat cortex is more sensitive to monovalent ions (Na^+, K^+) than to divalent cations (Ca^{++}, Mg^{++}). The [^{3}H]-tryptamine binding site was also shown to be a protein site which increases in number with age. The plasticity of the site was demonstrated by chronic administration of the selective and non-selective monoamine oxidase-inhibiting drugs [clorgyline and (-)-deprenyl, and phenelzine and tranylcypromine, respectively], and the tricyclic antidepressants imipramine, desipramine and clomipramine. The monoamine oxidase-inhibiting drugs induced a decrease in the [^{3}H]-tryptamine binding site density, while the tricyclic antidepressant drugs were without effect. Studies of the effects of chronic drug administration on tryptamine- and apomorphine-induced suppression of locomotor activity in the rat revealed that the behavioural effects of tryptamine did not correlate with changes in [^{3}H]-tryptamine binding site density, nor with functionally assessed changes in presynaptic dopamine D_2 receptor density. Acute studies revealed dose- and time-dependent locomotor suppression following tryptamine administration. The tryptamine-induced locomotor suppression was potentiated by clorgyline pretreatment but not by clomipramine pretreatment. Chemically, N-2-cyanoethyltryptamine acted as a prodrug for tryptamine, increasing levels of tryptamine both centrally and peripherally. Administration of N-2-cyanoethyltryptamine induced a dose- and time-dependent locomotor suppression which was not potentiated by pretreatment with clorgyline but was by clomipramine pretreatment. Alterations in endogenous neurotransmitter amine levels following N-2-cyanoethyltryptamine treatment differed from those observed following tryptamine treatment. These data suggest an action of this prodrug other than the specific delivery of tryptamine. A preliminary investigation of the efficacy of the N-propargyl analogue of tryptamine as a tryptamine prodrug was also conducted. This analogue induced an increase in brain tryptamine levels. Inhibition of monoamine oxidase by this compound may account for the observed neurochemical and behavioural effects.

This thesis is dedicated to my family:

Robert W. Mousseau,

Marlene Mousseau,

Michelle, Robert and Amy Mousseau-Schmidt,

and Corinne Brom,

and to two special women:

Loraine Verena Mousseau (née Fischer)

Apr. 09, 1931 - Jun. 07, 1990

and

Anna Marie Fischer (née Huber)

May 28, 1907 - Oct. 06, 1989.

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LIST OF ABBREVIATIONS

T	tryptamine
CET	N-2-cyanoethyltryptamine
PGT	N-2-propargyltryptamine
PEA	β-phenylethylamine
5-HT	5-hydroxytryptamine, serotonin
5-HIAA	5-hydroxyindole-3-acetic acid
5-MT	5-methyltryptamine
DA	dopamine
DOPAC	3,4-dihydroxyphenylacetic acid
HVA	homovanillic acid
NA	noradrenaline, norepinephrine
MAO	monoamine oxidase
AAD	L-aminoacid decarboxylase
GC-ECD	gas chromatography with electron capture detection
HPLC-ECD	high performance (pressure) liquid chromatography with electrochemical detection
*p*CPA	para-chlorophenylalinine
CLG	clorgyline
IMI	imipramine
DMI	desipramine, desmethylimipramine
TCP	(±)-tranylcypromine
PLZ	phenelzine, β-phenylethylhydrazine
DEP	(-)-deprenyl
CMI	clomipramine, chloroimipramine
FC	frontal cortex
HIPPO	hippocampus
HYPO	hypothalamus
CN	caudate nucleus
OT	olfactory tubercle

LIST OF ABBREVIATIONS
(continued)

min	minute
h	hour
l	litre
ml	millilitre
µl	microlitre (10^{-6} litre)
g	gram
kg	kilogram (10^{3} gram)
mg	milligram
ng	nanogram (10^{-9} gram)
mM	millimolar
µM	micromolar (10^{-6} molar)
nM	nanomolar (10^{-9} molar)
fmol	femtomole (10^{-15} mole)
K_D	dissociation constant (in nM)
B_{max}	maximum number of binding sites (in fmol mg^{-1} protein)
IC_{50}	concentration at which 50% of binding is inhibited
DHA	dihydroalprenolol
Tris	Tris buffer
ip	intraperitoneal
icv	intracerebroventricular
sc	subcutaneous

2 LITERATURE REVIEW

Introduction: Research into the etiology of psychiatric disorders such as depression benefitted greatly by the observation that iproniazid, a drug used in the treatment of tuberculosis, had mood-elevating properties demonstrated by euphoria and hyperactive behaviour (Selikoff *et al.*, 1952). During the same year it was discovered that iproniazid had a potent MAO-inhibiting effect (Zeller *et al.*, 1952), which subsequently led to reports of increases in brain monoamine [noradrenaline (NA) and serotonin (5-HT)] levels following administration of this drug (Brodie *et al.*, 1956). At approximately the same time (i.e. 1953), chlorpromazine was being marketed as a compound with therapeutic (neuroleptic) potential (see Bickel, 1977). A derivative of this compound, imipramine, although originally tested clinically for potential neuroleptic activity demonstrated a distinct antidepressant action (Kuhn, 1957). It was later shown that at least one of the modes of action of this drug was the inhibition of the uptake of NA into the nerve terminal thus resulting in an increased synaptic availability of this compound (Glowinski and Axelrod, 1964). The effects of these MAO-inhibiting and uptake-blocking drugs on brain 5-HT and NA availability formed the empirical basis for the biogenic amine hypothesis of depression (Schildkraut, 1965; Coppen, 1967). This hypothesis was further substantiated by observations that drugs which deplete these amines (e.g. reserpine) can cause depression (Klein, 1968). Put simply, this hypothesis states that a deficiency in the monoamines 5-HT and/or NA may account for depressive disorders.

Although this hypothesis provided the framework for investigating metabolic abnormalities of neurotransmitters in depressed patients, and for investigating the possible mechanisms of action of antidepressant drugs, doubts concerning the validity of this hypothesis arose following a number of observations: (a) biochemical changes occur almost immediately after drug administration; however, the time-course for mood reversal is days to weeks (Oswald *et al.*, 1972; Quitkin *et al.*, 1986; Post *et al.*, 1987), (b) some of the second generation

or novel antidepressants (e.g. iprindole and mianserin) are neither potent uptake blockers nor MAO inhibitors (Freeman and Sulser, 1972; Rosloff and Davis, 1974; Sanghvi and Gershon, 1975; Coppen *et al.*, 1976; Sulser *et al.*, 1978), and (c) cocaine, an effective amine uptake blocker, is not an effective antidepressant (Klein and Davis, 1969). In 1972, Ashcroft *et al.* proposed a modified amine hypothesis. They stressed that interactions between neuronal systems, as well as alterations of post-synaptic receptor sensitivity, should be considered in affective disorders. Changes at the receptor level are induced by tricyclic antidepressants, MAO-inhibiting drugs, as well as by the novel antidepressants (Charney *et al.*, 1981; Enna *et al.*, 1981). These effects are seen as either alterations in the function of second messenger systems (e.g. adenylate cyclase-catalyzed formation of cyclic adenosine 3',5'-monophosphate; cAMP) or as the decrease in density (down-regulation) of β-adrenergic or 5-HT_2 receptors (Baudry *et al.*, 1976; Sulser *et al.*, 1978; Peroutka and Snyder, 1980; Charney *et al.*, 1981). Although results from preclinical studies suggest antidepressants exert effects on neurotransmitter receptors within a time-course comparable to recovery from depression (Enna *et al.*, 1981; Coryell *et al.*, 1982; Sugrue, 1983), Katz *et al.* (1987) state that the clinical efficacy of antidepressant drugs cannot be attributed solely to changes in receptor sensitivity. Indeed, changes in somatic symptoms, such as sleep disorder, appetite loss, and cognitive function occur early during drug therapy, indicating a progression of pharmacologic effects which may initiate a series of events culminating in mood reversal, the latter coinciding with alterations in receptor sensitivity (Katz *et al.*, 1987). Although work on amines has concentrated on NA, dopamine (DA) and 5-HT, the brain also contains several other amines which, because of their low endogenous concentrations (<5 ng g^{-1} tissue range), have received limited attention. These "trace" amines, which include, among others, tyramine (TA; *m*- and *p*-isomers), octopamine (OA; *m*- and *p*-isomers), phenylethylamine (PEA) and tryptamine (T), have generally been assumed to be metabolic byproducts in the synthesis of the classical biogenic amines NA, DA or 5-HT (McGeer *et al.*, 1979; Boulton, 1984). As early as 1968, however, Dewhurst (1968a,b) had suggested

that these trace amines may be neurotransmitters or neuromodulators in their own right, and recent electrophysiological and neurochemical evidence seems to support this suggestion (Boulton, 1979; Boulton and Juorio, 1982; Jones, 1982d, 1983). The discovery of high affinity binding sites for many of these trace amines has further intensified research in this area (Hauger *et al.*, 1982; Kellar and Cascio, 1982; Perry *et al.*, 1982; see Greenshaw and Dewhurst, 1987 and van Nguyen and Juorio, 1989a for reviews).

2.A ANTIDEPRESSANT DRUGS

The serendipitous discovery that iproniazid (via its MAO blocking capability) and imipramine (via its amine uptake blocking ability) were effective antidepressants, combined with the observed mood-altering effects of reserpine and chlorpromazine, set the basis for neuropsychopharmacology as it stands today (see introduction to Section 2). Although electroconvulsive therapy (ECT) has been shown to elicit similar effects to antidepressant drug treatments (see Baker and Greenshaw, 1989), thus making it a highly effective treatment for depression (Brandon *et al.*, 1984), the present discussion will be limited to the use and actions of the MAO-inhibiting and tricyclic antidepressant drugs. Recent reviews on this subject are available (Blier and de Montigny, 1985; Dubovsky, 1987; Baker and Greenshaw, 1989; Baldessarini, 1989).

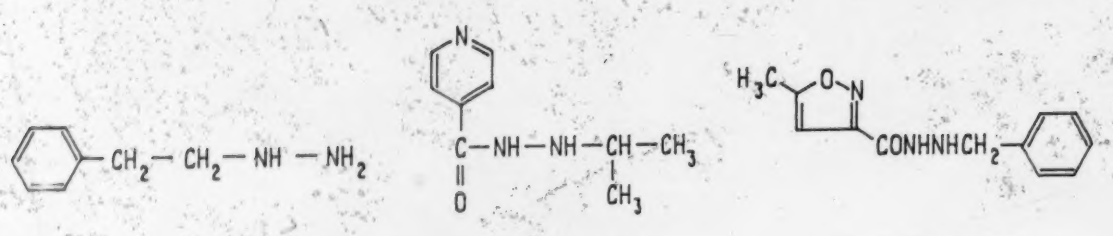
2.A.1 Monoamine Oxidase Inhibitors

Hare (1928) described an enzyme, present in rabbit liver, capable of oxidizing TA, which he called TA oxidase. Research into the actions of this enzyme, from both animal and plant sources, revealed its potential for oxidizing amine-containing compounds, and thus led to the name amine oxidase (Blaschko *et al.*, 1937). The present label, MAO, was given by Zeller (1951) who subdivided amine oxidases into MAO and diamine oxidase as a function of their substrates. MAO was further subdivided into MAO-A and -B on the merit of the affinity of either form for specific substrates (5-HT was designated as a preferential substrate for MAO-A, whereas benzylamine and PEA were designated as preferential substrates for

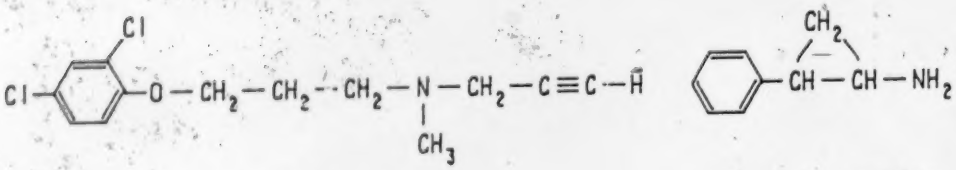
MAO-B). Clorgyline, an irreversible MAO inhibitor, was established, as a preferential type-A inhibitor following the observation that it inhibited the oxidation of 5-HT at nanomolar concentrations, whereas it inhibited the oxidation of benzylamine at micromolar concentrations (Johnston, 1968). (-)-Deprenyl had been shown earlier to exert what is now recognized as being type-B selective inhibition (Knoll *et al.*, 1965). By definition, MAO-A is the MAO isozyme sensitive to nanomolar concentrations of clorgyline, whereas MAO-B is that sensitive to nanomolar concentrations of (-)-deprenyl.

The relative proportion of brain MAO-A and MAO-B varies between species. In both man and rat MAO-A appears to be associated with the intraneuronal fraction (Fowler *et al.*, 1984; Stenstrom *et al.*, 1987). Whereas brain tissue from the laboratory rat possesses more MAO-A (55%) than MAO-B activity, the opposite is true for primates, including man, with MAO-B activity accounting for 70-85 % of the total brain MAO activity (Garrick *et al.*, 1979; Garrick and Murphy, 1980; Stenstrom *et al.*, 1987). The guinea pig brain displays an MAO-A/MAO-B ratio similar to that seen in man and might therefore prove to be a better model than the rat for the study of MAO-mediated metabolism (Azzaro *et al.*, 1985). In man, platelet MAO activity is used as an indicator of brain MAO activity and it has been proposed that the therapeutic efficacy of MAO-inhibiting drugs is reflected in their ability to inhibit MAO in platelets [≥85% inhibition of platelet MAO appears to be required for antidepressant activity] (Ravaris *et al.*, 1980). The neurochemical effects of MAO-inhibiting drugs may not, however, be limited to their MAO-inhibiting abilities. Chronic treatment with tranylcypromine has been shown to increase aromatic L-amino acid decarboxylase (AAD) activity in the rat, whereas phenelzine increases tryptophan (TRP) hydroxylase activity (Robinson *et al.*, 1979). It is not known whether the latter effect is a direct effect or a secondary effect due to decreased brain 5-HT levels resulting from decreased food (and hence tryptophan) ingestion. Phenelzine also inhibits γ-aminobutyric acid (GABA)- and alanine-transaminase activity *in vitro* (Popov and Matthies, 1969; Baker and Martin, 1989), suggesting an involvement of these two amino acids in the overall clinical profile of this

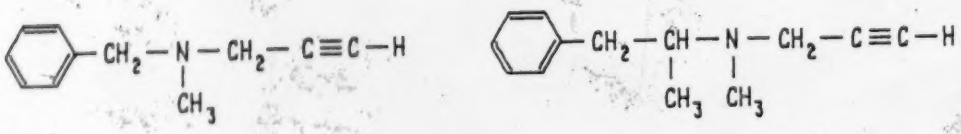
drug. In addition, both pargyline and phenelzine affect tyrosine metabolism by inhibiting tyrosine hydroxylase and tyrosine amino transferase activity, respectively (Mandell, 1975; Dyck and Dewar, 1986). Tranylcypromine and phenelzine have also been demonstrated to significantly increase the release of NA, DA and 5-HT from rat brain tissue preparations *in vitro* (Hendley and Snyder, 1968; Baker *et al.*, 1978, 1980a; Hampson *et al.*, 1986). It is now generally accepted that the main effect of MAO inhibitors is the increased availability of one or more of the biogenic amines NA, DA and 5-HT following blockade of their catabolism (Campbell *et al.*, 1979; Cohen *et al.*, 1985; O'Regan *et al.*, 1987). The effect of MAO-inhibiting drugs on other amines, specifically the trace amines *m*- and *p*-TA, PEA and T, suggested a potential etiological role for these trace amines in psychiatric disorders (Eccleston *et al.*, 1966; Dewhurst, 1968a,b; Philips and Boulton, 1979). In animal studies, the most consistent receptor-related findings, following treatment with MAO inhibitors, are the reduction of the cAMP response to NA and decreased β-adrenoceptor and 5-HT_2 receptor binding (see Heninger and Charney, 1987, and Baker and Greenshaw, 1989 for reviews). The MAO-inhibiting drugs most widely used in North America are the two hydrazines, phenelzine and isocarboxazid, and the non-hydrazines, tranylcypromine and pargyline (Dubovsky, 1987) [see Figure 1]. Available clinical data suggest that selective MAO-A inhibiting compounds, such as clorgyline, brofaromine and moclobemide, are most efficient in the treatment of depression (Baldessarini, 1984; Delini-Stula *et al.*, 1988). An important side effect of MAO-A inhibitors, however, is the TA-pressor effect. TA is a substrate for both MAO-A and -B. Blockade of type-A MAO results in increased TA levels which may ultimately act on NA nerve endings to cause hypertensive reactions (Baldessarini, 1989). In fact, the incidence of hypertensive responses is five times greater with tranylcypromine than phenelzine treatment (Blackwell *et al.*, 1967), perhaps as a result of tranylcypromine's additional ability to affect the release and uptake of NA at nerve terminals (Hendley and Snyder, 1968; Baker *et al.*, 1978; Hampson *et al.*, 1986). The reversible MAO-A-inhibiting compounds, brofaromine and moclobemide, exhibit only weak TA-pressor effects and may



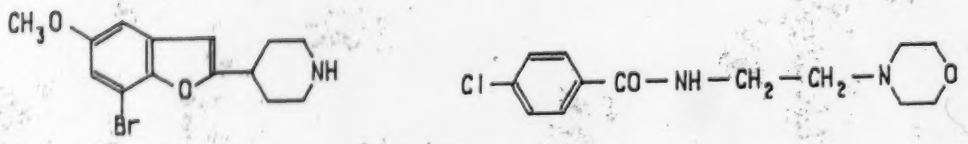
Phenelzine Iproniazid Isocarboxazid



Clorgyline Tranylcypromine



Pargyline Deprenyl



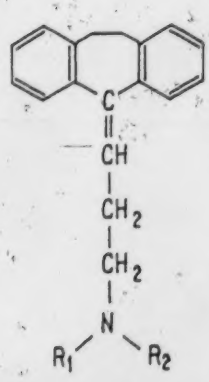
Brofaromine Moclobemide

Figure 1: **Structures of hydrazine (top) and non-hydrazine (bottom) monoamine oxidase inhibitors.**

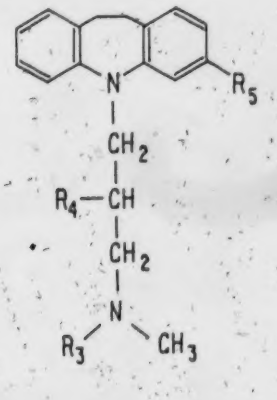
therefore prove to be useful as antidepressant drugs (Delini-Stula *et al.*, 1988). In addition, the metabolism of MAO inhibitors is an important determinant of side-effects. Phenelzine is metabolized to PEA-like compounds by MAO itself (Clineschmidt and Horita, 1969). Both (-)-deprenyl and tranylcypromine have been reported to be metabolized to amphetamine-like compounds which, in the case of the latter MAO inhibitor, may be responsible for the occasional instance of apparent addiction (Reynolds *et al.*, 1980; Karoum *et al.*, 1983; Briggs *et al.*, 1990).

2.A.2 Tricyclic Antidepressants

The imipramine and amitriptyline series of tricyclic antidepressant drugs block the active uptake of biogenic amines (i.e. NA, 5-HT and to a lesser extent DA) at nerve terminals, resulting in the increased synaptic availability of these amines (Richelson, 1984). The tricyclic antidepressants have been useful therapeutic agents in depression, bulimia, panic anxiety, posttraumatic-stress disorder and obsessive-compulsive disorder (Montgomery, 1980; Goodman and Charney, 1985; Zohar and Insel, 1987). The parent tertiary amines (i.e. imipramine and amitriptyline) block primarily the uptake of 5-HT, whereas the secondary amine metabolites (i.e. desipramine and nortriptyline) affect primarily NA uptake (Klein *et al.*, 1980) [see Figure 2]. The hydroxylated metabolites of these compounds exert similar effects to the corresponding parent drugs on 5-HT and NA uptake, but the added hydroxyl group often makes them more hydrophilic than the parent compounds and thus more readily excreted (Glenn and Taska, 1984). As with the MAO-inhibiting drugs, the most common feature of chronic tricyclic antidepressant treatment is a decrease in β-adrenoceptor binding-site density and altered 5-HT function (see Goodman and Charney, 1985 and Baker and Greenshaw, 1989 for reviews). These are by no means the only effects observed with these compounds. Hrdina (1987) demonstrated, using MAO inhibitors and tricyclic and novel antidepressants, that the affinity of the [^{3}H]-imipramine binding site was decreased, although the number of binding sites remained unchanged ([^{3}H]-imipramine



$R_1 = CH_3$; $R_2 = CH_3$	Amitriptyline
$R_1 = CH_3$; $R_2 = H$	Nortriptyline
$R_1 = H$; $R_2 = H$	Desmethylnortriptyline



$R_3 = CH_3$; $R_4 = H$; $R_5 = H$	Imipramine
$R_3 = H$; $R_4 = H$; $R_5 = H$	Desipramine
$R_3 = CH_3$; $R_4 = H$; $R_5 = Cl$	Clomipramine
$R_3 = H$; $R_4 = H$; $R_5 = Cl$	Desmethylclomipramine
$R_3 = CH_3$; $R_4 = CH_3$; $R_5 = H$	Trimipramine

Figure 2: **Structures of the amitriptyline (top) and imipramine (bottom) series of tricyclic antidepressant drugs.**

binds to a site closely associated with the 5-HT uptake site; Langer and Briley, 1981). In contrast, increased densities of $GABA_B$- (Lloyd *et al.*, 1985) and α_1-adrenergic binding sites (Campbell and McKernan, 1982) have been indicated, whereas a decrease in DA D_1 binding site density was found (Klimek and Nielsen, 1987), following chronic tricyclic antidepressant treatment.

The side-effects of tricyclic antidepressants are varied, ranging from anticholinergic effects, drowsiness and hypotension to tremor, tachycardia and (rarely) seizures and photosensitivity (Abramowicz, 1980). The most common complaints during tricyclic antidepressant therapy are the anticholinergic effects (dry mouth, blurred vision, constipation, *etc.*) and orthostatic hypotension (Ananth, 1983), and these are generally associated with tertiary tricyclic antidepressant drug use.

The significance of a gradual loss of β-adrenoceptor sensitivity during pharmacotherapy is unclear. Indeed, Blier and de Montigny (1985) proposed that β-blockade *per se* is not an adequate explanation for the antidepressant effects of both MAO inhibiting and tricyclic antidepressant drugs. This suggestion was based on: (a) the observation that amphetamine and chlorpromazine, neither of which are effective antidepressants, both decrease β-adrenoceptor binding site density (Baudry *et al.*, 1976; Schultz, 1976); (b) the possibility that the observed subsensitivity of β-adrenoceptor responses might be a compensatory effect, via negative feedback mechanisms, for the increased synaptic availability of NA, with no net change in the gain (i.e. the overall postsynaptic response to the stimulation of the receptor population) (Blier and de Montigny, 1985); (c) the fact that the β-adrenergic antagonist propranolol may sometimes induce depression in certain patients (Waal, 1967; Petrie *et al.*, 1982); and (d) the observation that some of the novel antidepressants (i.e. mianserin and zimelidine) do not affect β-adrenoceptor binding, but do decrease cAMP formation (Mishra *et al.*, 1980).

The feasability of a NA/5-HT link in brain with regard to receptor change has been discussed

by Baker and Greenshaw (1989). ECT-induced potentiation of 5-HT-mediated behaviours is abolished following noradrenergic lesions (Green and Deakin, 1980). Furthermore, Asakura *et al.* (1987) have shown that intrasynaptic 5-HT levels are not important for the initial decrease of β-receptors, although they are important for maintaining β-receptor down-regulation. Finally, chronic desipramine treatment does not induce cortical β-adrenoceptor down-regulation if 5-HT pathways leading to the cortex are lesioned with 5,7-dihydroxytryptamine (5,7-DHT) (Manier *et al.*, 1987). This latter result is in contrast, however, with the finding of Stockmeier and Kellar (1988) which demonstrated that ECT decreases β-adrenoceptor density despite 5,7-DHT lesions of 5-HT neurons. This discrepancy may be explained by the recent observation by Manier *et al.* (1987) and Gillespie *et al.* (1988) that β-adrenoceptors exist in high- (70% of the total cortical β-adrenoceptor population) and low-affinity conformations. These sites are pharmacologically differentiated by their affinity for the β-agonist isoproterenol and have been studied using [^{3}H]-dihydroalprenolol ([^{3}H]-DHA) as the radioligand. The [^{3}H]-DHA binding site having a high affinity for isoproterenol is clearly a β-adrenoceptor. The identity of the site with low affinity for isoproterenol remains unclear. It is possible that both sites are β-adrenoceptors in different affinity conformations; however, it is also possible that the low-affinity site is a recognition site for a neurotransmitter other than NA (Stockmeier and Kellar, 1988). In fact, Riva and Creese (1989a,b) have proposed that the second site with an affinity for [^{3}H]-DHA might be the 5-HT$_{1B}$ receptor as the selective 5-HT$_{1B}$ receptor agonists RU 24969 and CGS 12066B both competed for 15-20% of [^{3}H]-DHA binding in the nanomolar range. Drugs selective for other 5-HT receptor subtypes only inhibited [^{3}H]-DHA binding at higher concentrations (Riva and Creese, 1989a). Thus the conflicting results reported by Manier *et al.* (1987) and Stockmeier and Kellar (1988) (see above) may in fact indicate differential effects of the respective antidepressant treatments on specific receptor types which are not revealed due to a lack of sensitivity of the radioligand. Aulakh *et al.* (1988) have suggested that the role of 5-HT in β-adrenoceptor regulation may be limited to the regulation of the

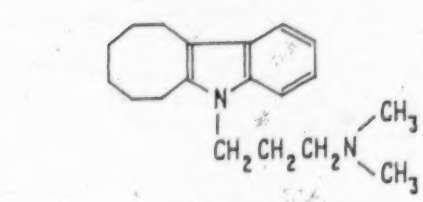
low-affinity conformation β-adrenoceptor. It is obvious that a possible NA/5-HT link with regard to receptor regulation, and consequently antidepressant efficacy, remains contentious, although such a link may be revealed given a more selective β-adrenoceptor radioligand. Riva and Creese (1989a,b) have demonstrated that the β-adrenoceptor antagonist radioligand [^{3}H]-CGP-12177 is much more selective for β-adrenoceptors than is [^{3}H]-DHA and may therefore elucidate the effects of antidepressant treatments on the adrenergic system. The effects of antidepressant drugs on 5-HT receptors are discussed in Section 2.B.2.

Although antidepressants other than the MAO-inhibitors and the tricyclic antidepressants exist, they will not be discussed in great detail in this review. Certain aspects of these drugs are, however, of interest. The "novel" or "second-generation" antidepressants [see figure 3] differ from the classical antidepressants as they do not have the fused tricyclic structure, nor are they effective MAO inhibitors. Unlike the classical antidepressants, which affect for the most part both the NA and 5-HT systems, the mechanisms of action of the novel antidepressants vary between selective effects on *either* the NA or 5-HT system, selective potent DA effects, and mixed DA and NA *or* 5-HT effects (for a review see Pohl and Gershon, 1983). Besides their therapeutic efficacy, these drugs also generally exhibit much less anticholinergic activity than the tricyclic antidepressants, and in certain cases virtually lack any such activity (Baldessarini, 1983, 1989; Pohl and Gershon, 1983). This latter point makes them very attractive for the treatment of affective disorders. The proposed cholinergic hypothesis of affective disorders which alleges that depressive and manic phases are marked by hyper- and hypocholinergic states, respectively (see Dilsaver, 1986) appears to suffer by the findings that the novel antidepressants virtually lack anticholinergic effects. More work is evidently required in this area of study.

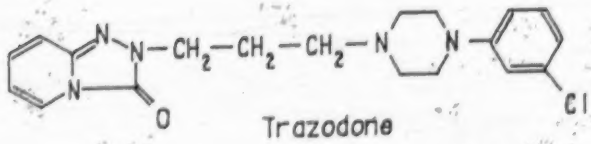
2.B 5-HYDROXYTRYPTAMINE (SEROTONIN; 5-HT)

2.B.1 Detection and Distribution of 5-HT

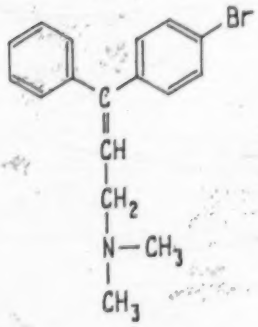
An endogenous vasoconstrictor substance, originally referred to as "vasotonin", was isolated



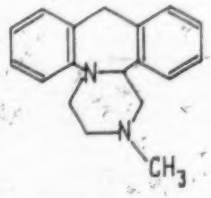
Iprindole



Trazodone



Zimelidine



Mianserin

Figure 3: Structures of some 'novel' or 'second-generation' antidepressant drugs.

successfully from serum and appropriately named serotonin (Rapport *et al.*, 1948). Serotonin may have been observed in the periphery as early as 1932 by Eros, but it was not until 1952 that this substance was shown to exist in enterochromaffin cells of the intestinal mucosa (Erpsamer and Asero, 1952). The chemical structure of serotonin was found to be the ring-hydroxylated analogue of T, hence the name 5-hydroxytryptamine (5-HT). 5-HT was first detected in the CNS by Twarog and Page (1953) but it was not until Falck *et al.* (1962) refined the fluorescence technique used by Eros (1932) that specific 5-HT-containing cell bodies were identified in rat brainstem (Dahlström and Füxe, 1965). Nine nuclei, labeled B1-B9, were found to contain 5-HT, two of which, the dorsal (B6 and B7) and median (B8) raphé nuclei, contained most of the 5-HT-producing neurons of the midbrain. These structures had been observed earlier by Ramon y Cajal (1911), who noticed nuclei located in the midbrain raphé which received afferents from the forebrain and which had large and branched neurons, projecting both rostrally and caudally. The reduction in forebrain 5-HT following destruction of raphé cells (Anden *et al.*, 1966) strengthened the observation that most ascending 5-HT-containing neurons had their origins in the midbrain raphé area (Füxe, 1965) [see Figure 4]. These findings and the earlier discovery that 5-HT could be formed in neurons (Heller *et al.*, 1962) suggested that 5-HT might be involved in neurotransmission. The synthesis of 5-HT is now recognized to result from hydroxylation of the precursor, TRP, via TRP hydroxylase, to 5-hydroxytryptophan (5-HTP) followed by decarboxylation, via AAD, to 5-HT (Brown *et al.*, 1980; see Figure 5). The concentration of 5-HT in the brain is in the range of ng g^{-1} wet weight tissue and is dependent on the availability of TRP (Fernstrom, 1983). The affinity of TRP-decarboxylase for 5-HTP is high (Lovenberg *et al.*, 1962), resulting in much larger quantities of 5-HT than of T being formed (see Section 2.C). In contrast to T, which is believed to be catabolized by both forms of MAO, 5-HT is preferentially catabolized by the A form of this enzyme (Neff *et al.*, 1974). The original belief that the raphé nuclei contained only 5-HT cell bodies has changed following work which demonstrated that, of the total number of cell bodies in the dorsal raphé nucleus and

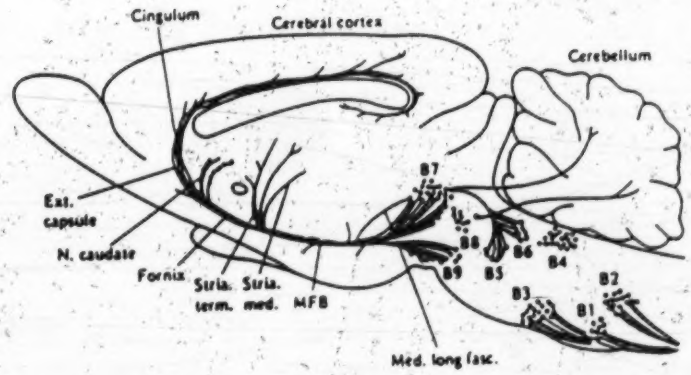


Figure 4: Neuronal pathways for 5-HT in the rat brain. Ext. capsule: external capsule; N. caudate: nucleus caudate; Stria. term.: striatum terminalis; Stria. med.: striatum medianus; Med. long fasc.: median longitudinal fascicle; MFB: median forebrain bundle. Taken with permission from Cooper *et al.* (1986).

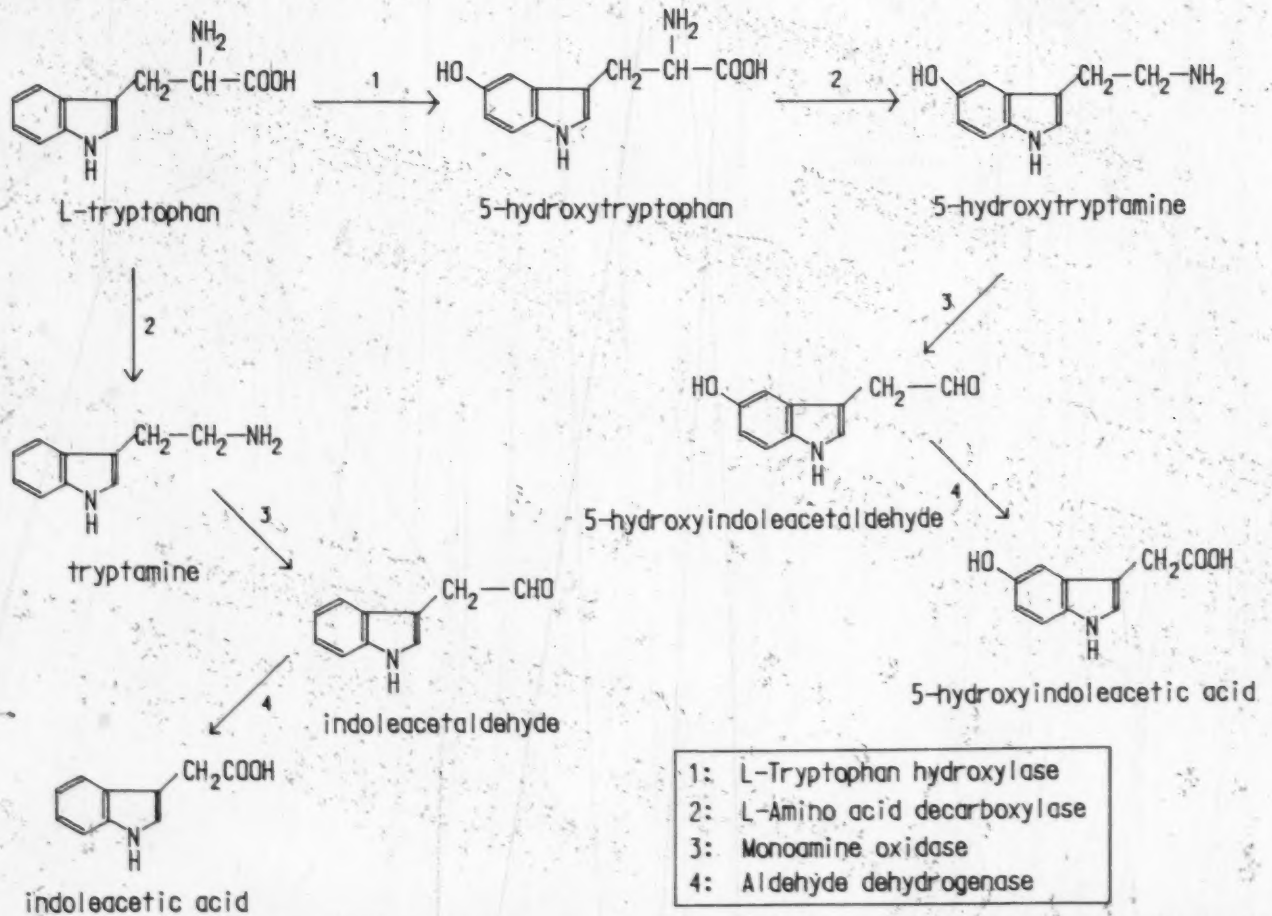


Figure 5: **Biosynthetic and degradative pathways of tryptamine and 5-hydroxytryptamine. L-Tryptophan hydroxylase requires tetrahydrobiopterin (as a co-factor) for proper function, whereas L-amino acid decarboxylase requires pyridoxal phosphate (as a co-factor) for proper function.**

the nucleus raphé pallidus, only 50% comprise 5-HT cell bodies (Halaris *et al.*, 1976; Moore, 1981). In fact, the dorsal raphé nucleus also contains DA, NA, cholecystokinin, leu- and met-enkephalin, vasoactive intestinal peptide and substance P, whereas the median raphé nucleus also contains DA (Steinbusch and Nieuwenhuys, 1983). The raphé nuclei display both ascending (rostrally projecting) and descending (caudally projecting) fibres. The B1-B3 nuclei have axons which terminate in the cervical and lumbar enlargements and in the sacral segment of the spinal cord (Jacobowitz, 1978). B4-B9 (the pontine and mesencephalic raphé nuclei) give rise to the ascending 5-HT tracts, with the majority of neurons originating in the dorsal and median raphé nuclei (Azmitia, 1978). The ascending fibres are contained mainly in a large fibre tract known as the median forebrain bundle (MFB). The MFB consists of both ascending and descending fibres from many different neurotransmitter systems, these being arranged systematically in a dorso-ventral fashion. Histochemical fluorescence examination of the rat brain revealed that, within the MFB, catecholamine fibres occupied the dorsal portion, whereas 5-HT fibres occupied the ventral portion, the latter being divided into ventral and lateral bundles (Ungerstedt, 1971).

2.B.2 5-HT Receptors and Effects on Behaviour

Receptors: 5-HT receptors (5-HT_M and 5-HT_D) were described and differentiated by Gaddum and Picarelli (1957). The 'M' and 'D' nomenclature originated from the compounds which were observed to antagonize contractile effects on guinea pig ileum mediated by these receptors, i.e. Morphine and Dibenzylline, respectively (see Bradley *et al.*, 1986 and Glennon, 1990 for reviews on the function and nomenclature of 5-HT receptors). Peroutka and Snyder (1979), basing their findings on radioligand binding studies, demonstrated the existence of the 5-HT_1 and 5-HT_2 binding sites. [^{3}H]-5-HT, [^{3}H]-lysergic acid diethylamide (LSD: a partial 5-HT agonist) and other 5-HT agonists were shown to bind with nanomolar affinity to the 5-HT_1 site, whereas [^{3}H]-spiperone, [^{3}H]-LSD and other 5-HT antagonists bound with nanomolar affinity to the 5-HT_2 site. The 5-HT_2 recognition sites and the '5-HT_D'

receptors were soon shown to be the same entity (see Hoyer, 1988). Although the 5-HT_3 site was tentatively suggested to be the '5-HT_M' receptor (Richardson *et al.*, 1985; Bradley *et al.*, 1986), recent evidence (Buchheit *et al.*, 1985; Mawe *et al.*, 1986) points to the existence of distinct sites (see Fozard, 1987). Drug tools for the pharmacological characterization of 5-HT receptors and radioligand binding studies have led to the identification and characterization of eight 5-HT receptors subtypes, all of which have been demonstrated to alter cellular function and/or produce physiological responses (Leysen *et al.*, 1982; DeVivo and Maayani, 1986; Conn *et al.*, 1986; Yocca *et al.*, 1987; Glennon *et al.*, 1987; Murphy and Bylund, 1988; Julius *et al.*, 1988; Schoeffter *et al.*, 1988; Hoyer and Neijt, 1988; Leonhardt *et al.*, 1989; Bockaert *et al.*, 1990). As the literature on this subject is extremely prolific and complex, a summary of the relevant points is given in Tables 1 and 2. To complicate matters further, the existence of a number of 5-HT_3 receptor subtypes has been suggested (see Hoyer, 1988).

Behaviour: The most prominent behaviour attributed to 5-HT in rodents is the so-called '5-HT syndrome' which is a characteristic behavioural pattern that can be elicited by a variety of 5-HT precursors, releasers or receptor agonists and potentiated by MAO-inhibiting drugs. This 'syndrome' is characterized by a myoclonic motor syndrome that in the rat consists of behaviours such as resting tremor, rigidity, reciprocal forepaw treading, hindlimb abduction, Straub tail and lateral head-weaving (Grahame-Smith, 1971; Jacobs, 1976). This syndrome is considered to be present in an all-or-none fashion if rats simultaneously exhibit at least four of the six component signs mentioned above. Certain evidence suggested that the 5-HT-induced behavioural syndrome may be spinally mediated. Jacobs and Klemfuss (1975), by systematically transecting rat brains at different levels and then injecting TRP and an MAO inhibitor, demonstrated that the 5-HT syndrome could be elicited in rats with only the pons, medulla and spinal cord left intact. In addition, Deakin and Green (1978) demonstrated, following the depletion of spinal cord 5-HT, an enhancement of the 5-HT syndrome presumably due to supersensitive spinal 5-HT receptors. The exact role of 5-HT

Receptor	Site(s)-action	Comments
'5-HT_1-like'		
8-OH-DPAT	5-HT_{1A}-agonist	[^{3}H]8-OH-DPAT radioligand; affinity for 5-HT transporter
dipropyl-5-carboxamidotryptamine	5-HT_{1A}-agonist	highly selective; poor brain penetration
(±)MDL 72832	5-HT_{1A}-agonist/antagonist	stereoselectivity at 5-HT_{1A} sites
buspirone	5-HT_{1A}-agonist/antagonist	dopamine receptor ligand; metabolized to 1-pyrimidinylpiperazine
ipsapirone	5-HT_{1A}-agonist/antagonist	metabolized to 1-pyrimidinylpiperazine
spiperone	5-HT_{1A}-antagonist	non-selective [5-HT $_2$; α_1]
spiroxatrine	5-HT_{1A}-agonist/antagonist	non-selective [D_2; opiate]
TFMPP	5-HT_{1B}-agonist	
CGS 12066B	5-HT_{1B}-agonist	
iodo-cyanopindolol	5-HT_{1B}-antagonist	^{125}I-labelled cyanopindolol radioligand; non-selective [β]
(-)-21-009	5-HT_{1B}-antagonist	non-selective [β]
mesulergine	5-HT_{1C}-antagonist	[^{3}H]mesulergine radioligand; non-selective [5-HT_2; D_2]
mianserin	5-HT_{1C}-antagonist	non-selective [5-HT_2]
rauwolscine	5-HT_{1D}-ligand	stereoselective interaction; non-selective [α_2]
(-)-pindolol	5-HT_{1A} + 5-HT_{1B}-antagonist	non-selective [β]; partial agonist at 5-HT_{1B} sites
cyanopindolol	5-HT_{1A} + 5-HT_{1B}-antagonist	non-selective [β]; partial agonist at 5-HT1B sites
RU 24924	5-HT_{1A} + 5-HT_{1B} + 5-HT_{1D}-agonist	antagonist on rat fundic strip
5-carboxamidotryptamine	5-HT_{1A} + 5-HT_{1B} + 5-HT_{1D} - agonist	impressive potency at each site
metergoline	non-selective - antagonist	
methiothepin (metitepine)	non-selective - antagonist	
AH 25086	'5-HT_1-like' - agonist	receptor remains to be defined - but not 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}
GR 43175	'5-HT_1-like' - agonist 5-HT_{1E}	known agonists and antagonists have low affinity for this site; human cortex

continued on next page

5-HT$_2$		
α-methyl-5-HT	agonist	similar affinity for 5-HT$_{1C}$ sites
DOB	agonist	4-methyl derivative (DOM) also active
methyserigide	antagonist	'5-HT$_1$-like' partial agonist
ketanserin	antagonist	poorly selective; [^{3}H]ketanserin radioligand
ritanserin	antagonist	allosteric modulator

5-HT$_3$		
2-methyl-5-HT	agonist	
phenylbiguanide	agonist	
MDL 72222	antagonist	high selective; no affinity for ileum 5-HT$_3$ receptor
ICS 205-930	antagonist	highly selective
BRL 24924	antagonist	highly selective; stimulant effects on ileum
BRL 43694	antagonist	highly selective
GR 38032F	antagonist	highly selective
zacopride[b]	antagonist	highly selective

5-HT$_4$[c]		
zacopride	agonist	high affinity in guinea pig hippocampal membranes and fetal mouse colliculi neurons
cisapride	agonist	see above
BRL 24924	agonist	see above

Table 1: Drug tools for 5-HT receptor pharmacology. Adapted from Fozard (1987) with additions from a- Leonhardt *et al.* (1989), b- Pinkus *et al.* (1989) and c- Bockaert *et al.* (1990). (±)**MDL 72832**, 8-[4-(1,4-benzodioxan-2-ylmethylamino)butyl]-8-azaspiro(4,5)decane-7,9-dione; **TRMPP**, 1-3(3-trifluoromethylphenyl)piperazine; **GCS12066B**, 7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrolo(1,2-a)quinoxaline; **(-)21-009**, (-)-4(3-ter-butyl-amino-2-hydroxypropoxy)-indol-2-carbonic acid isopropylester; **RU 24924**, 5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole; **AH 25086**, 3-(2-aminoethyl)-1H-indole-5-(*N*-methyl) acetamide; **GR 43175**, 3-(2-dimethylaminoethyl)-1H-indole-5-(N-methyl)methane sulphonamide; **DOB**, 4-bromo-2,5-dimethoxyphenyl-isopropylamine; **MDL 72222**, 1αH,3α,5αH-tropan-3-yl)3,5-dichlorobenzoate; **ICS 205-930**, (3α-tropanyl)-1H-indole-3-carboxylic acid ester; **BRL 24524**, (+)-(endo)-4-amino-5-chloro-2-methoxy-*N*(1-azabicyclo(3,3,1)non-4-ylbenzamide; **BRL 43694**, (endo)-*N*-[9-methyl-9-azabicyclo-(3,3,1)non-3-yl]-1-methyl-indazole-3-carboxamide; **GR38032F**, 1,2,3,9-tetrahydro-9-methyl-3[C2-methyl- 1H-imidazol-1-yl]-4H-carbazol-4-one.

5-HT_{1A}
Adenylate activation (via G_s) or inactivation (via G_i) CA1 and raphe cell inhibition Canine basilar artery contraction Classical 5-HT syndrome Coupled to K^+ channels in hippocampus Facilitation of ejaculation and/or seminal emissions Hypotensive effects Hypothermia Somatodendritic autoreceptor
5-HT_{1B}
Inactivation of adenylate cyclase activity Not present in human brain Presynaptic autoreceptor
5-HT_{1C}
Abundant in choroid plexus Stimulation of phosphoinositide turnover
5-HT_{1D}
Closely parallel (in human brain) distribution of 5-HT_{1B} sites in rat brain High affinity for ergot derivatives and tryptamine Inhibition of adenylate cyclase activity
5-HT_{1E}
In human brain Interact with GTP-binding protein
5-HT_{2}
5-HTP- or mescaline-induced head twitches Classical 'D' receptor Contraction of bronchial and vascular smooth muscle Discriminative cue properties Down-regulation following antidepressant and ECT treatment Hyperthermia Neurophysiologic excitation of neuronal firing Phosphoinositide turnover Platelet shape changes and aggregation Rat forepaw edema Smooth muscle prostacyclin synthesis Tryptamine-induced seizures
5-HT_{3}
Agonists block migraine and chemotherapy-induced emesis Antagonists involved in therapeutic treatment of psychosis and drug addiction Membrane cation channels
5-HT_{4}
Activation of adenylate cyclase

Table 2: Proposed functional correlates of 5-HT binding site subtypes. Adapted from Peroutka (1986) with data added from Fozard (1987), Aghajanian *et al.* (1988), Schoeffter *et al.* (1988), Derkach *et al.* (1989), Leonhardt *et al.* (1989), Tricklebank (1989) and Bockaert *et al.* (1990).

in this syndrome remains unclear, however, as a number of studies failed to find any significant correlations between the occurrence of the 5-HT syndrome and high brain concentrations of 5-HT (Grahame-Smith, 1971; Foldes and Costa, 1975; Magyar *et al.*, 1978). It appears that a subtype of the 5-HT receptor, specifically the 5-HT_{1A} receptor, actually mediates the 5-HT-induced behavioural syndrome (Tricklebank *et al.*, 1984; Yamada *et al.*, 1988). Recent evidence also suggests that the 5-HT_2 receptor might mediate certain components of the 5-HT syndrome, particularly the head-shake and the head-twitch, as blockade of the 5-HT_2 receptor clearly antagonizes these behaviours (Peroutka *et al.*, 1981; Leysen *et al.*, 1982; Colpaert and Janssen, 1983). Sloviter *et al.* (1978) and Schlosberg and Harvey (1979) observed that neither catecholamine antagonists nor catecholamine depletion affected the syndrome evoked by 5-methoxy-N,N-dimethyl-T (5-MeO-DMT) or 5-HTP, although other studies indicate that the opposite is true, implicating not only the catecholamines but the trace amine T as well (see Section 2.C.5).

Dysfunctions in the 5-HT system have been associated with the etiology of depression (see Section 2.A). The control of a variety of specific behaviours, many of which are altered in depression, has been attributed to 5-HT function (Barchas and Usdin, 1973). These include mood, appetite, sexual activity, circadian rhythms, sleep, pain and thermoregulation (for review see Meltzer and Lowy, 1987).

The involvement of 5-HT in the expression of mood has been demonstrated through alterations of dietary TRP content. A TRP-free diet resulted in normal males in significantly elevated scores on the depression scale of the Multiple Affect Adjective Checklist (Young *et al.*, 1985), whereas TRP-loading produced subjective mood elevations ("mellow" and "high" feelings) in volunteers when compared to a placebo control group (Smith and Prockop, 1962; Charney *et al.*, 1982). In general, antidepressants have variable effects on 5-HT_1 sites, whereas many of these agents can down-regulate 5-HT_2 sites (Peroutka and Snyder, 1980; Leonard, 1988). There is, however, some evidence for the involvement of 5-HT_{1A} receptors in antidepressant efficacy. The 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-prop-

ylamino) tetralin (8-OHDPAT; Middlemiss and Fozard, 1983) exerts what appears to be an antidepressant effect in laboratory animals, while the 5-HT_{1A} agonist buspirone appears to act as an antidepressant in the clinical setting (Kennett *et al.*, 1987a,b).

Increased 5-HT activity is usually correlated with decreased appetite (Blundell, 1984), although some depressed patients, particularly those with seasonal affective disorder, crave carbohydrates (Rosenthal *et al.*, 1984). In the laboratory animal, 5-HT activity appears to be inversely related to feeding since the 5-HT_{1A} receptor agonist 8-OHDPAT elicits hyperphagia in free-feeding rats (Dourish *et al.*, 1985), whereas this same agonist attenuates fenfluramine-induced anorexia, presumably by a stimulatory action on the 5-HT autoreceptor (Dourish *et al.*, 1988). Montgomery *et al.* (1988) have suggested that 8-OHDPAT-induced hyperphagia may simply be the result of an enhanced chewing response elicited by the drug, as they observed an increase in wood block chewing by 8-OHDPAT treated rats deprived of food but failed to find an increase in intake of various liquid diets by similarly treated rats. Stimulation of postsynaptic 5-HT_{1B} receptors, on the other hand, seems to account for the hypophagic effect induced by RU 24969 (Kennett and Curzon, 1988).

In depression, changes in sexual behaviour may be an indirect result of altered 5-HT function. A decreased libido, paralleled by an inhibitory effect on the release of gonadal hormones, has been attributed to enhanced 5-HT function (McEwen and Parsons, 1982). As this does not fit the 5-HT hypothesis of depression (e.g. a decreased synaptic availability of 5-HT), it has been suggested that the decrease in libido observed during depression might be a secondary result of the disease, i.e. following loss of interest or motor retardation (Meltzer and Lowy, 1987). Although 5-HT_{1A} agonists were found to inhibit sexual activity in male mice (Svensson *et al.*, 1987), these compounds generally enhance sexual behaviour in the male rat (Olivier *et al.*, 1988) and decrease it in the female rat (Croissant *et al.*, 1988). 5-HT_{1B} receptors have also been implicated in the sexual activity of rats. In contrast to the effects seen with 5-HT_{1A} agonists, 5-HT_{1B} agonists facilitate sexual behaviour in the female rat and

inhibit it in the male rat (Mendelson and Gorzalka, 1988; Olivier *et al.*, 1988). *l*-Kynurenine and kynuramine (products of TRP metabolism) facilitate lordosis in the female rat (Mendelson *et al.*, 1987). In addition, kynuramine exhibits a high affinity for the [^{3}H]-T binding site (Charlton *et al.*, 1984), thus indicating a possible involvement of this binding site in at least an aspect of sexual behaviour.

Disturbance of circadian rhythms is a feature observed in certain patients with affective disorder (Wehr and Goodwin, 1981). Utilization of 5-HT is lowest when rats are inactive [i.e. during the day; Bruinink *et al.*, 1983] and highest at night (Hery *et al.*, 1972). Such circadian variations in 5-HT levels and 5-HT receptors may mediate the circadian rhythm of certain hormones, most importantly those of the hypothalamic-pituitary-adrenal axis (Meltzer and Lowy, 1987). Abnormalities in the function of the components of this axis, specifically in depressive illness, results in hypersecretion of cortisol (Carpenter and Bunney, 1971; Carroll, 1976) and a blunted thyrotropin response following thyrotropin-releasing hormone (TRH) administration (Prange *et al.*, 1972). Cortisol secretion exhibits a diurnal rhythm, with its peak in the morning hours (Gwirtsman *et al.*, 1982), while TRH release is inhibited by 5-HT (Ettigi and Brown, 1977). 5-HT has also been shown to exhibit a diurnal rhythm (Hery *et al.*, 1972). In addition, the suprachiasmatic nucleus, a structure known to be the pacemaker in rat circadian rhythms, has a profuse 5-HT innervation (Meltzer and Lowy, 1987). An altered sleep cycle, resulting in insomnia, is also a common symptom of depression. Inhibition of 5-HT synthesis by blockade of TRP-hydroxylase with para-chlorophenylalanine (*p*CPA; Koe and Weissman, 1966) produces insomnia, whereas TRP generally improves sleep (Hartmann, 1983; Sallanon *et al.*, 1983). The decreased latency to the rapid eye movement (REM) phase of the sleep cycle is the characteristic most often observed in major depression (Kupfer, 1978) and it has been shown that low doses of 8-OHDPAT increase the latency to REM sleep, as well as decrease the number of wakenings, whereas high doses increase wakefulness in the rat (de St. Hilaire-Kafi *et al.*, 1987). 5-HT$_2$ antagonists consistently suppress REM sleep but exert varied effects on non-REM sleep

(Pastel, 1988).

The availability of TRP, and subsequently that of 5-HT, may influence the perception of pain. Approximately half of the patients diagnosed with depression report an increase in pain sensitivity (von Knorring *et al.*, 1984). Treatment with *p*CPA resulted in hyperalgesia (Tenen, 1967; Messing and Lytle, 1977), whereas intrathecal administration of 5-HT to rats increased the latency for tail-flick [a response in laboratory animals used to measure pain sensitivity] (Larson, 1985). This analgesic effect of 5-HT is thought to be related to an interaction with endogenous opiate systems. One of the major sites of analgesic action by opiates is on the neurons of the periaqueductal and periventricular gray (PAG and PVG, respectively) regions of the brain (Anderson and Proudfit, 1981). These neurons interact with 5-HT neurons of the median raphé nucleus which project to the dorsal horn of the spinal cord. Thus, opiates activate neurons in the PAG and PAV areas that subsequently activate the bulbospinal 5-HT system to produce analgesia. In fact, analgesia resulting from microinjections of morphine into the PAG region is attenuated by intrathecal injection of the 5-HT antagonist methysergide (Yaksh, 1979; Yaksh and Tyce, 1979). Recently, the 5-HT_3 antagonist, ICS 205-930, has been found to effectively block pain when applied to a blister base on the human forearm, thus implicating this receptor in nociception (Richardson *et al.*, 1985).

Thermoregulation is now known to be involved intimately with 5-HT function. Increases in body temperature and shifts in the time of nocturnal minimal temperature have been observed in patients with affective disorder (Avery *et al.*, 1982). Gudelsky *et al.* (1988) have demonstrated hypothermia and hyperthermia in rats following stimulation of 5-HT_{1A} and 5-HT_2 receptors, respectively. Thermoregulatory aspects of 5-HT function are discussed in greater detail in Section 2.C.4.

As can be seen from this brief overview, the actions of 5-HT are many and complex, perhaps as a result of its diverse distribution throughout the brain and resultant interactions with a

number of other neurotransmitter systems. Its structural congener T also exerts a diversity of effects which are discussed in the next section.

2.C TRYPTAMINE (T)

T is one of many compounds, including PEA, TA, OA and synephrine among others, which had been neglected in early etiological studies of psychiatric disorders. The low endogenous levels (low nmol g^{-1} concentrations) of these amines [hence the label "trace amine" (Usdin and Sandler, 1976)] in tissues and the fact that they share synthetic and catabolic enzymes with the classical amines (see Figure 5) fueled the confusion which led to their being referred to as "metabolic accidents" or "false transmitters" (McGeer *et al.*, 1979; Boulton, 1984). As early as the 1960's, however, a functional deficiency of these amines, PEA and/or T in particular, was proposed as a possible etiological factor in depression (Dewhurst and Marley, 1965; Dewhurst, 1968a,b; Sabelli and Mosnaim, 1974). Interest in the trace amines has heightened with the advent of highly specific and sensitive mass-spectrometric techniques allowing their accurate quantification, as well as the characterization of their metabolic pathways [see Boulton and Juorio (1982), and Jones (1982d) for reviews]. Although these compounds are interesting in their own right, the focus of this thesis is T and further discussion will therefore center on this indolic trace amine.

2.C.1 Detection of Tryptamine

Using a spectrofluorimetric technique, Hess *et al.*(1959) found T to be below the limits of detection for the assay (i.e. <100 ng g^{-1}) in brain tissue of untreated rat, dog or guinea pig. Treatment with the amino acid TRP or concurrent treatment with TRP and an MAO inhibitor reportedly led, however, to detectable levels (1-3 μg g^{-1}) in guinea pig brain (Hess *et al.*, 1959; Eccleston *et al.*, 1966). Following the same procedure, Green and Sawyer (1960) subsequently reported brain T levels of 32 ng g^{-1} in the untreated rat while levels of 100 ng g^{-1} were claimed by Hess and Doepfner (1961). It was later shown that this spectrofluorimetric technique was flawed in that TRP was carried over into the extracted T phase thereby

giving false estimates of T content (Eccleston *et al.*, 1966). The addition of ion exchange chromatography to the fluorimetric assay allowed for the detection of T in guinea pig brain only after combined TRP/MAO inhibitor treatment (Eccleston *et al.*, 1966). Martin *et al.* (1972) reported levels of up to 180 ng g^{-1} in dog (following 5 mg kg^{-1} isocarboxazid) and untreated steer brain, although T was still not detectable in the untreated rat brain. Saavedra and Axelrod (1972a), Knott *et al.* (1974), and Martin *et al.* (1974), using a radioenzymatic assay, all found T levels in rat brain to be approximately 20 ng g^{-1}, whereas Snodgrass and Horn (1973) using a procedure involving derivatization with a dansyl reagent, found whole rat brain concentrations to be 70 ng g^{-1}. A much lower estimate of 0.5 ng g^{-1} for whole rat brain T concentration was given by Philips *et al.* (1974) with the use of a more specific and sensitive mass-spectrometric technique. This estimate was later confirmed by Warsh *et al.* (1977b), Artigas and Gelpi (1979) and Calverley *et al.* (1980), and more recently by Artigas *et al.* (1984), Juorio and Durden (1984), Larson and Dalo (1986), and Peura *et al.* (1988).

2.C.2 Metabolism of Tryptamine

The synthesis of brain T occurs by decarboxylation of TRP via the action of the enzyme AAD. This pathway is now well established (see Figure 5). TRP, although a non-essential amino acid, is available from the diet and readily crosses the blood-brain barrier for use by the brain (Oldendorf, 1971a,b). It is possible that a portion of measured brain T arises from sources other than the brain since T is also readily absorbed into the brain from the blood (Lemberger *et al.*, 1971; Oldendorf, 1971a; Oldendorf and Braun, 1976). T and AAD activity have been reported in such peripheral tissues as the kidney and liver (Rosengren, 1960; Awapara *et al.*, 1962). Increases in brain T have been observed following the administration of TRP, the effect being potentiated by concurrent treatment with a MAO-inhibiting drug (Saavedra and Axelrod, 1973, 1974; Snodgrass and Horn, 1973; Warsh *et al.*, 1979; Juorio and Durden, 1984). Blockade of AAD results in a depletion of endogenous T and a blockade of the increase in T observed following either TRP (Saavedra and Axelrod, 1974) or pargyline

treatment (Juorio and Durden, 1984). Inhibition of 5-HT synthesis with *p*CPA treatment resulted in increased T levels (Saavedra and Axelrod, 1973, 1974; Snodgrass and Horn, 1973; Marsden and Curzon, 1974; Snodgrass and Iversen, 1974), presumably because of an increase in the availability of the precursor, TRP. Juorio and Durden (1984) failed, however, to find such a change in T levels following *p*CPA administration. These authors explained this discrepancy on the basis of assay sensitivity and specificity. In the earlier studies the measured levels of endogenous T were about two orders of magnitude greater than values measured using mass-spectrometry (see Section 2.C.1). It may well be that the apparent increase in T was due to other compounds being detected by the earlier procedures. It has been demonstrated recently that neither acute nor chronic *p*CPA treatment affects [^{3}H]-T binding site density (Graham and Langer, 1987). Brain T concentrations, although dependent on TRP concentrations (Warsh *et al.*, 1979), are also kept at their physiological levels by the affinity of AAD for TRP. Under physiological conditions, the affinity of AAD for TRP is very low, with K_m levels of approximately 1.0 - 1.5 x 10^{-2} M (Christensen *et al.*, 1970; Ichyamma *et al.*, 1970), resulting in levels of T a thousand-fold lower than those of 5-HT (Artigas and Gelpi, 1979). The presence of substantial quantities of TRP and AAD in many monoaminergic neurons may be an indication that the synthesis of T could occur in any of these neurons (see Juorio and Paterson, 1990 for a review). The existence of *p*CPA-resistant neurons (i.e. neurons which exhibit unaltered TRP-induced fluorescence following *p*CPA treatment) in rat median raphé nuclei (Aghajanian and Asher, 1971) and spinal cord (Björklund *et al.*, 1970; 1971; 1976) led the latter authors to the conclusion that certain CNS neurons, distinct from 5-HT neurons, were capable of producing T. Neurons present in the spinal cord (Jaeger *et al.*, 1983) and in the brainstem (Jaeger *et al.*, 1984b) which were neither catecholaminergic nor serotonergic (as attested by the lack of tyrosine hydroxylase and 5-HT), were found to contain AAD. These cells were labelled "D" cells by Jaeger *et al.* (1984a) following the nomenclature set forth by Dahlström and Fuxe (1964). The recent observation that astrocytes do not exhibit AAD activity (Juorio *et al.*, 1987b)

strengthens the assumption that the synthesis of T may well occur intraneuronally in monoaminergic neurons as well as in "specific" trace amine neurons which do not synthesize catecholamines or 5-HT. Cells which stain for indoleamine-immunoreactivity following *p*CPA/MAO inhibitor treatment have also been observed in embryonic rat dorsal myelencephalon (Bieger, 1985). Furthermore, although an association between T and the DA system has been demonstrated using measures of binding parameters (van Nguyen *et al.*, 1989; see Section 2.C.6), and measures of brain T concentrations following electrolytic or neurotoxin-induced lesions of the substantia nigra (Juorio and Greenshaw, 1986; Juorio *et al.*, 1987a; see Section 2.C.4), studies simultaneously using monoclonal antibody anti-DA and antiserum anti-T showed that T and DA are not co-localized in the same neurons, at least not in the substantia nigra and midbrain raphé nuclei (Geffard *et al.*, 1987; Dabadie *et al.*, 1990). Accurate determination of the distribution of trace amines at the level of the single cell, using antibody reactivity techniques, has been questioned by Paterson *et al.* (1990) based on the apparent lack of specificity of the antibody to the target trace amine. These authors argue, using the example of antibodies for PEA, that cross-reactivity of the [trace-amine] antibodies to NA, DA, 5-HT, and L-amino acids, might lead to erroneous results. Paterson *et al.* (1990) further state that determination of distribution patterns of trace amines to a finer resolution, i.e. to the scale of smaller brain nuclei, might be feasible using a new gas chromatography-mass spectrometry technique exhibiting a sensitivity of approximately 20 pg (D.A. Durden, 1990: unpublished observation quoted in Paterson *et al.*, 1990).

The major route of catabolism for T is one of enzymatic inactivation, which is a pathway common to many of the biogenic amines. Sequential action by MAO (see Section 2.A) and aldehyde dehydrogenase results in the formation of indole-3-acetic acid (IAA) via the aldehyde (Weissbach *et al.*, 1961; Wu and Boulton, 1973). The aldehyde/IAA ratio significantly increased if sodium thiosulfate was included in the isolation procedures, indicating that the aldehyde is quickly metabolized to IAA under normal conditions (Wu

and Boulton, 1973). This result has since been repeated using rat brain (Warsh *et al.*, 1977a) and human cerebrospinal fluid (Young *et al.*, 1980). While T has been claimed to be oxidized principally by MAO-A (Fuller, 1972; Bieck *et al.*, 1983, 1984) or by MAO-B (Houslay and Tipton, 1974), it has also been suggested that T is a substrate for both forms of the enzyme (Neff and Yang, 1974; Mantle *et al.*, 1975; Durden and Philips , 1980; Suzuki *et al.*, 1981; Sullivan *et al.*, 1986). Non-selective MAO inhibition with iproniazid (Eccleston *et al.*, 1966; Juorio and Durden, 1984), pargyline (Marsden and Curzon, 1974; Warsh *et al.*, 1977b; Philips and Boulton, 1979; Durden and Philips, 1980), phenelzine (Baker *et al.*, 1985, 1988b) or tranylcypromine (Philips and Boulton, 1979; Hay *et al.*, 1982; Baker *et al.*, 1984b, 1985; Nazarali *et al.*, 1985; Coutts *et al.*, 1987) resulted in substantial increases in rat brain T concentrations. This effect was observed with and without concurrent TRP treatment. MAO inhibitor-induced increases in T levels are also known to occur in a variety of species, including mouse, rat, rabbit, cat, dog and the domestic fowl (Snodgrass and Horn, 1973; Boulton *et al.*, 1975; Sloan *et al.*, 1975; Danielson *et al.*, 1976; Juorio, 1976; Warsh *et al.*, 1977b; Juorio and Durden, 1984). Selective MAO inhibition with clorgyline (selective for MAO-A) or (-)-deprenyl (selective for MAO-B) yielded contrasting results as clorgyline induced a 3-fold increase in brain T, whereas (-)-deprenyl had no effect (Philips and Boulton, 1979). An *in vitro* study by Fuller (1972) indicated that the reversible MAO-A inhibitor harmaline was more effective at impeding T deamination than was pargyline (specific MAO-B inhibitor at low doses). Bieck *et al.* (1984) also found similar results when comparing the effects of the reversible MAO-A inhibitor CGP 11-305A with those of (-)-deprenyl on the urinary excretion of T in healthy volunteers. In these latter cases the predominant form of MAO involved in T metabolism might depend on the location of the two forms of the enzyme (Fowler *et al.*, 1984; Stenstrom *et al.*, 1985). In rat brain MAO-A is known to be localized primarily in the synaptosomal fraction, whereas MAO-B mainly exists extrasynaptosomally (Oreland *et al.*, 1983; Fowler *et al.*, 1984). Suzuki *et al.* (1981) have reported that rat liver MAO-A has a lower K_m value and a higher V_{max} for T than does

MAO-B. Comparable observations were made with human liver, although kidney cortex and medulla, and cerebrocortical tissue displayed equivalent contributions of the two forms of MAO toward T metabolism (Sullivan *et al.*, 1986). Substrate specificities of the two forms of MAO in human brain regions varied, with the ratio of activity due to MAO-A *vs* MAO-B being greatest in the cerebral cortex and least in the medulla oblongata (Sullivan *et al.*, 1986). Kinetic measurements in brain tissue indicated that the half-life for T was 0.28 min (in contrast to 77 min for 5-HT) (Durden and Philips, 1980). While this is in agreement with an estimate of 0.66 min, which these same authors calculated using the synthesis rate of Warsh *et al.* (1979), and the endogenous T concentration reported by Philips [unpublished, cited in Boulton (1976)], a later study by Juorio and Durden (1984) gave a slightly higher estimate of 1.6 min (rat spinal cord). The turnover rate of T [0.24 nmol g^{-1} per h (brain), Durden and Philips, 1980; 0.04 nmol g^{-1} per h (spinal cord), Juorio and Durden, 1984] had been known, for a number of years, to be relatively rapid. This was indicated by its large accumulation following inhibition of MAO (Wu and Boulton, 1973; Marsden and Curzon, 1974; Philips and Boulton, 1979) as well as by the fact that T injected by the intracerebroventricular (*icv*) route is rapidly catabolized by MAO (Meek *et al.*, 1970; Jones, 1982d). Although T is rapidly catabolised and exists in minute absolute amounts in brain tissue, its rapid synthesis allows for quantities, per unit time, which are comparable to those reported for 5-HT and DA (Wu and Boulton, 1973; Durden and Philips, 1980).

The catabolism of T by MAO and aldehyde dehydrogenase is by no means the sole route of inactivation of this compound, albeit it is a major one. A minor portion of the aldehyde is known to be reduced to indole-3-ethanolamine by aldehyde reductase (Susilo *et al.*, 1988). N-methyltransferase, the enzyme involved in methylation of T, has been shown to exist in human brain, lung and blood (Axelrod, 1962; Mandell and Morgan, 1971; Mandel *et al.*, 1972; Saavedra and Axelrod, 1972b; Wyatt *et al.*, 1973; Barchas *et al.*, 1974), as well as in rat brain (Barker *et al.*, 1980). Increased activity of this enzyme, leading to abnormal quantities of the hallucinogenic N-methyl and N,N-dimethyl derivatives of T, was proposed

as a possible factor in the pathogenesis of psychiatric disorders such as schizophrenia (see Section 2.C.7). Small amounts of N-methyl- (1%) and N,N-dimethyl-T (0.1-0.2%) were detected following *icv* injection of T in the rat (Wu and Boulton, 1973; Boulton and Baker, 1974) and these T derivatives have also been demonstrated in human blood (Walker *et al.*, 1979). In addition to the methylation of indoleamines, this enzyme has been shown to be important in the formation of β-carbolines (Barchas *et al.*, 1974). The skeleton of the β-carboline molecule has been suggested as deriving from a condensation reaction of T and acetaldehyde (Honnecker and Rommelspacher, 1978; Bloom *et al.*, 1982; Collins, 1985; Susilo and Rommelspacher, 1987). These last authors have also shown that a β-carboline, 1,2,3,4-tetrahydro-1-methyl-β-carboline-1-carboxylic acid, formed following the *icv* injection of T and pyruvic acid, in rats pretreated with an MAO inhibitor, might be an intermediate in an alternate synthetic pathway for β-carbolines, leading to harmalan, harman and tetrahydroharman (Rommelspacher *et al.*, 1985; Susilo and Rommelspacher, 1987). The reaction of pyruvic acid and T or 5-HT was already known to yield similar tetrahydroharman derivatives (Hahn *et al.*, 1935; Collins *et al.*, 1982). It is of interest to note that the distribution, in brain, of 1,2,3,4-tetrahydro-β-carbolines, or tryptolines as they are otherwise known, appears to have very little similarity with that of T, the presumed biosynthetic precursor (Peura *et al.*, 1989). Other minor catabolic pathways involve the formation of 6-hydroxytryptamine (Jepson *et al.*, 1962; Lemberger *et al.*, 1971), thiazolidine-4-carboxylic acid derivatives [via the condensation of indole-3-acetic acid with L-cysteine] (Susilo *et al.*, 1988) and kynurenine-like derivatives via indoleamine 2,3-dioxygenase (Hirata and Hayaishi, 1972; Hayaishi, 1976). The action of N-acetyltransferase, which is present in rat brain homogenates (Paul *et al.*, 1974; Voisin *et al.*, 1984; Namboodiri *et al.*, 1987), on indoleamines appears to be limited to 5-HT (Chan and Ebadi, 1981), as it exerts no effect on endogenous T (Wu and Boulton, 1973). It has, however, been reported as being involved in T metabolism in the crayfish (Dubbels and Elofsson, 1989). T is apparently not a biosynthetic precursor for 5-HT (Douglas, 1980) and

to date, the conversion of T to 5-HT has only been observed in cell suspension cultures of *Peganum harmala* [wild rue] (Sasse *et al.*, 1982; Courtois *et al.*, 1988).

2.C.3 Regional and Subcellular Distribution of Tryptamine

Regional Distribution: Philips *et al.* (1974, 1980), using mass-spectrometry, found regional differences in T concentrations within the rat brain, with the striatum and hippocampus exhibiting the highest concentrations (2.94 ng g^{-1} and 1.45 ng g^{-1}, respectively) and the brainstem having the lowest (0.24 ng g^{-1}). Comparable values for regional concentrations in mouse brain were reported by Juorio and Durden (1984). A similar trend in regional distribution was observed by Knott *et al.* (1974) and Snodgrass and Horn (1973), although the latter authors, having extended their analyses to include the spinal cord, observed the highest levels of T in this structure. As mentioned previously, the T assay performed by these last two research groups was not as specific and sensitive as the mass-spectrometry assay employed by Philips *et al.* (1974) and thus their results remain questionable. A comparative regional study of the distribution of T and 5-HT by Knott *et al.* (1974) indicated that the distribution of these two indoleamines are not the same. Although 5-HT levels were highest in the hypothalamus and relatively homogeneous throughout the rest of the brain, the levels of T were highest in striatal and hypothalamic tissue. These authors proposed that the discrepancy in the distribution patterns of T and 5-HT indicated an independent existence of these two amines. This hypothesis was substantiated by electrolytic lesion studies of the raphé nuclei which resulted in reduced accumulations of approximately 70% and 30% in 5-HT and T brain concentrations, respectively (Knott *et al.*, 1974; Marsden and Curzon, 1974). A later study by Juorio and Greenshaw (1985) demonstrated a similar effect wherein electrolytic lesions of the midbrain raphé nuclei resulted in unaltered T levels for up to two weeks post-lesion, whereas 5-HT levels were reduced by 50%. Electrolytic lesions of the substantia nigra, however, resulted in reduced accumulations of striatal T and DA concentrations to approximately 50% of control values, indicating a dependence of T

concentrations on the integrity of the substantia nigra (Juorio and Greenshaw, 1986). Intranigral lesions with the catecholamine-selective toxin, 6-hydroxydopamine (6-OHDA), and the indoleamine-selective toxin, 5,7-DHT, indicated a similar pattern of distribution for T (Juorio *et al.*, 1987a). 5,7-DHT-induced lesions had no effect on striatal T, whereas a reduced accumulation in T levels was observed following 6-OHDA-induced lesions. These results suggest that decreases in striatal T might be a result of a loss of nigral DA-containing neurons. Alternatively, there might be specific T-containing neurons which are affected by 6-OHDA but not by 5,7-DHT (Juorio *et al.*, 1987a), these neurons perhaps being the "D" neurons detected by Jaeger *et al.* (1984a: see Section 2.C.2).

The presence of T in human brain tissue and cerebrospinal fluid has been confirmed by various research groups (Martin *et al.*, 1972; Saavedra and Axelrod, 1973; Philips *et al.*, 1978; Young *et al.*, 1980). The study by Philips *et al.* (1978) reflected their earlier findings with rat brain (Philips *et al.*, 1974) in that human caudate nucleus contains the highest concentrations of T. Using high-resolution mass-spectrometry for the determination of T, levels below the limits of sensitivity of the assay were reported in whole brain of domestic fowl, the optic lobes of the octopus and ganglia of the locust (Juorio, 1976; Juorio and Philips, 1976; Robertson, 1976). The starfish's arm nerve is the only tissue studied to date which exhibits substantial concentrations of T. Levels of 1251 ng g^{-1} have been reported; however, since levels of 5-HT in this species are virtually non-existent, it may be that T is the important indoleamine in echinoderms (Juorio and Robertson, 1977).

Subcellular Distribution: T formed in brain is most likely neuronal in origin (Jones, 1982d). The subcellular fractionation of rat brain revealed T to be associated with the "crude mitochondrial" fraction, and particularly with the synaptosomal subfraction (Boulton and Baker, 1975). Furthermore, Cascio and Kellar (1983) and Perry *et al.* (1988) demonstrated that [^{3}H]-T binding sites were also associated with the synaptosomal fraction. Snodgrass and Iversen (1974) showed that, in the rat spinal cord slices, [^{3}H]-T formed from [^{3}H]-TRP can be released in a Ca^{++}-dependent manner by depolarizing stimuli (i.e. high [K^+] and

electrical stimulation), leading them to the assumption that T was being released from neurons. The release was abolished with high [Mg^{++}], low [Ca^{++}] and the presence of colchicine in the incubation medium. Hery *et al.* (1979), studying [^{3}H]-T release *in vivo* from cat caudate nucleus, found a similar decrease in the release of [^{3}H]-T in low [Ca^{++}] conditions, but failed to observe any changes in release with high [K^+]. Dyck (1984a,b; 1989) stated that both endogenous and [^{14}C]-T release only occurred spontaneously as it could not be stimulated by a variety of stimuli including K^+, unlabeled T, 5-HT, (+)-amphetamine and veratridine, although [^{3}H]-5-HT release was increased. This had been demonstrated earlier by Osborne (1980) using bovine retina. The uptake of T seems to be predominantly a passive diffusional process rather than a result of an active high affinity uptake. Born *et al.* (1972) and Costa *et al.* (1977) failed to demonstrate an active and saturable uptake process in human platelets, while Ross and Ask (1980) observed similar accumulations of T at 0°C and 37°C in rat hypothalamic preparations. In contrast to these findings, a more recent study by Segonzac and coworkers (1984) demonstrated a saturable and Na^+-dependent uptake of [^{3}H]-T in rabbit platelets as well as in human platelets (Segonzac, Tateishi and Langer, unpublished observations, quoted in Segonzac *et al.*, 1984). Jones and Broadbent (1982a) found the uptake of T by rat cortical tissue to proceed via a similar high affinity temperature- and Na^+-dependent mechanism. Dyck (1984a,b), on the other hand, found the *in vitro* uptake of T to consist mainly of a diffusional process, at 37°C, although a small active uptake was present between 1.0-1.5°C. This active uptake, presumably a Na^+-dependent carrier-mediated process requiring metabolic energy, was inhibited by low extracellular Na^+, dinitrophenol, ouabain and cocaine, all of which inhibited the active uptake of [^{3}H]-5-HT. As well, specific 5-HT uptake blockers (zimelidine, norzimelidine, fluoxetine and citalopram) affected T uptake, although to a lesser extent than that observed for 5-HT uptake (Dyck, 1984a,b). This latter effect confirmed previous observations in rat cortical and hypothalamic tissue (Ross and Ask, 1980; Jones and Broadbent, 1982a) and rabbit platelets (Segonzac *et al.*, 1984). In the caudate nucleus,

however, the effect was reversed, with T uptake being affected more than 5-HT, presumably as a result of a greater contribution of the diffusional process to T uptake than to 5-HT uptake (Dyck, 1984b). Jones and Broadbent (1982a) found effective T uptake blockade with fluoxetine but failed to find such an effect with zimelidine. Fluoxetine failed to enhance the behavioural effects of combined T and 5-HT treatment in mice, although it enhanced 5-HT-induced behaviours (Larson and Wilcox, 1984). These latter authors also demonstrated that T enhanced the behavioural effects of 5-HT and suggested, based on these data, that T enhanced the effects of 5-HT by competing for the same uptake site, thus prolonging the synaptic availability of 5-HT (Larson and Wilcox, 1984).

The claim that T is stored in granules originated following observations that T levels decreased in animals having received reserpine treatment (Lemberger *et al.*, 1971; Snodgrass and Horn, 1973). Storage of T is suggested by the observation that T is accumulated by rat brain slices (Wu and Boulton, 1973) and by a crude particulate fraction (Shah *et al.*, 1973) and is present in the synaptosomal subcellular fraction of brain (Boulton and Baker, 1975). Stimulus-induced release of T from rat spinal cord slices also diminished in reserpinized animals (Snodgrass and Iversen, 1974), although a more recent study by Juorio (1982) resulted in an unexplained increase in mouse brain T following vesicular disruption. The observation that prior reserpine treatment resulted in increased [^{3}H]-T binding in rat cortical and hippocampal tissue also indicates a possible storage of T in reserpine-sensitive vesicles (Cascio and Kellar, 1986), although this effect was not noticed in other brain regions. In addition, Delini-Stula and Radeke (1985) observed an increase in T-induced behaviours in reserpine-treated rats (22 h pretreatment), indicating postsynaptic supersensitivity, presumably as a result of disruption of T storage. In contrast to these findings, no changes in T concentrations were observed by a number of research groups studying reserpinized rat (Saavedra and Axelrod, 1973, Marsden and Curzon, 1974) and dog (Sloan *et al.*, 1975) brain. Meek *et al.* (1970) demonstrated rapid catabolism of T following *icv* injection, thus suggesting a lack of vesicular storage of this amine. Segonzac *et al.* (1984) found that

differences in the V_{max} for [^{3}H]-T and [^{3}H]-5-HT uptake into rabbit platelets disappeared following reserpine treatment, indicating a likely vesicular storage for 5-HT but not for T. These authors stated that the difference in vesicular storage might explain why the V_{max} of [^{3}H]-5-HT uptake is higher than that of [^{3}H]-T and why this difference disappears following the disruption of vesicular integrity by reserpine. Although the subject of possible vesicular storage of T remains unclear, Jones (1982d) suggests that the lack of sensitivity and/or specificity of the assay techniques used in most of the earlier studies should be taken into account in the interpretation of the results.

2.C.4 Interaction of Tryptamine with Other Transmitter Systems

5-HT: T inhibits the *in vitro* active uptake of 5-HT in rat hypothalamus (Horn, 1973), striatum (Baker *et al.*, 1977, 1980b) and cortex (quoted in Jones, 1982d). T-induced inhibition of 5-HT uptake has also been demonstrated in human platelets (Born *et al.*, 1972; Airaksinen *et al.*, 1980), although experiments using [^{14}C]-T in rat synaptosomes failed to show any evidence of an interaction between T and the 5-HT uptake carrier (Ross and Ask, 1980). Recent evidence (Langer *et al.*, 1983; Segonzac *et al.*, 1985) that T modifies the dissociation kinetics of platelet [^{3}H]-imipramine binding supported an earlier suggestion that T and 5-HT exhibit an allosteric interaction at the level of the [^{3}H]-imipramine recognition site in brain (Sette *et al.*, 1983) and platelets (Wennogle and Meyerson, 1983; Langer *et al.*, 1983). Fuxe and Ungerstedt (1968) demonstrated a T-induced release of cytosolic 5-HT using fluorescence histochemistry while a similar effect has been observed in striatal prisms and synaptosomal preparations (Baker *et al.*,1977, 1980b). Under fixed volume conditions, T increases the release of endogenous 5-HT (Robinson and Marsden, 1984). Recent findings indicate that the K^+-induced release of 5-HT from superfused midbrain raphé nuclei (Ennis and Cox, 1982), hypothalamic (Cox *et al.*, 1981b; Cox and Ennis, 1982) and cortical slices (Mounsey *et al.*, 1982) is attenuated by T. The difference between the effects of T on 5-HT release under fixed volume and superfused conditions is

that the former involves addition of T to the preparation at the beginning of incubation, thus allowing for accumulation in presynaptic neurons and evoked 5-HT release (Robinson and Marsden, 1984). Under superfusion conditions, T flows continually through the system, which according to Robinson and Marsden (1984) prevents the uptake and accumulation of T in neurons.

Ernst *et al.* (1961) proposed that T-induced behavioural effects in cats were a result of antagonism of central 5-HT systems. Mutual antagonism of T and 5-HT has also been demonstrated in immature and adult fowl (Dewhurst and Marley, 1965; Marley and Nitisco, 1975; Jones, 1981). Neurochemically, T has been shown to have a moderate affinity for 5-HT binding sites (IC_{50} values ranging from 80 to 300 nM: Bennett and Snyder, 1976; Whittaker and Seeman, 1978; Seeman *et al.*, 1980). In addition, 5-HT receptor antagonists (i.e. metergoline and methysergide) have been shown to block certain T-induced physiological responses, thus suggesting the possibility of 5-HT receptor-mediated T responses. Three of these physiological processes, namely thermoregulation, neuronal firing rate and vasoconstriction, will presently be discussed to illustrate the action of T on the 5-HT system. Behavioural effects of T will be discussed in Section 2.C.5.

Although much attention has been directed toward an involvement of hypothalamic 5-HT in thermoregulation (see Jacob and Girault, 1979 for review), there is recent evidence of a possible role for T. Administration of T or its precursor, TRP, has been reported to produce hypothermia in mice (Dooley and Quock, 1976) and hyperthermia in rabbits (Quock and Weick, 1978) and domestic fowl (Marley and Nitisco, 1975). An "endogenous indoleamine" other than 5-HT has been implicated in the heat production pathways of sheep (Bligh *et al.*, 1979). Reports of differential effects of intrahypothalamic injections of T (hyperthermia) and 5-HT (hypothermia) in the rat, as well as the differential blockade by indoleamine receptor antagonists, suggested that these responses were mediated by two different receptor populations (Quock and Weick, 1978; Cox *et al.*, 1981a,b, 1983). Cyproheptadine significantly reduced the response to 5-HT but not T, whereas methysergide and metergoline

affected the T response but not that of 5-HT. It was hypothesized that T might inhibit the release of 5-HT from nerve terminals, thus inhibiting a heat-loss pathway (Cox *et al.*, 1983). Quock and Weick (1978) have also shown that the hyperthermic effects of T are potentiated by the 5-HT uptake blocker, fluoxetine. Yamada *et al.* (1987b) have demonstrated that methysergide, which has an affinity for 5-HT_1 and 5-HT_2 receptors, attenuated hypothermia induced by *icv* T in mice, whereas the 5-HT_2 receptor antagonist ketanserin was without effect. This finding and the observation that 8-OHDPAT, a specific 5-HT_{1A} receptor agonist, induces hypothermia in mice and rats (Goodwin *et al.*, 1985; Hjorth, 1985) led to the conclusion that T-induced hypothermia is mediated by the 5-HT_1 receptor (Yamada *et al.*, 1987b). Although the IC_{50} values for T at the 5-HT_1 and 5-HT_2 binding sites are relatively high [66.7 nM and 3.85 μM, respectively; Locock *et al.*, 1985], it is possible that under circumstances leading to increased T levels (e.g. following MAO-inhibitor treatment), an effect of T on the 5-HT_1 receptor could be attained. It is interesting to note that, whereas methysergide has been shown to abolish T-induced thermoregulatory effects, T-induced hyperalgesia is reported to be potentiated by methysergide (Larson, 1983, 1985). Finally, the T analogue 5-methoxytryptamine has also been proposed as a potential candidate for modulation of 5-HT-induced thermoregulation as it exhibits similar effects to T on heat regulation (unpublished data quoted in Cox *et al.*, 1983) and is also found in rat hypothalamus (Green *et al.*, 1973).

Curtis (1962) failed to discern any T-induced effect on the firing of spinal cord interneurons. Later studies, however, found potent T-induced depressant effects on the firing rate of single cells in the lateral geniculate nucleus (Curtis and Davis, 1962), cortical cells of the cat (Krnjevic and Phillis, 1963), molluscan neurons (Gerschenfeld and Stefani, 1966), ventral lateral geniculate, dorsal raphé nucleus and amygdala (Aghajanian and Haigler, 1975) and cortical cells in the rat (Jones and Boulton, 1981; Jones, 1982a,b). Jones and Boulton (1981), studying the effects of T and 5-HT on single cortical neurons, observed two types of interactions. On cells where 5-HT elicited depressant responses, weak iontophoretic

applications of T potentiated these responses. On cells where 5-HT elicited excitatory responses, similar applications of T either abolished the excitation or replaced it with depressant responses. The latter observation was also noticed in cortical cells exhibiting excitation following electrical stimulation of the median raphé nucleus (Jones, 1982b,c). Two phases in cell response were observed; (a) a short-latency inhibitory response and (b) late excitatory and inhibitory responses (Jones, 1982a,b; Jones and Broadbent, 1982b). The late phase was enhanced by TRP loading and attenuated by *p*CPA treatment, whereas the early phase was enhanced by combined *p*CPA and TRP treatment (Jones and Broadbent, 1982b). These latter results indicate a T-mediated early inhibition and a late 5-HT-mediated excitation/inhibition. The suggestion that both excitatory and inhibitory receptors coexist on the same neuron may explain the differing actions of both these indoleamines on individual neurons (Jones and Boulton, 1981). When 5-HT is excitatory, the specific blockade of the excitatory receptors by T reveals the depressant effect, whereas in the case where 5-HT is inhibitory the blockade by T of the excitatory receptors would potentiate the 5-HT-mediated depressant effects. The depressant effects were shown subsequently to act through a metergoline-sensitive receptor (Jones, 1981). A rostrally projecting pathway with its origins in the midbrain raphé nuclei and containing an indoleamine other than 5-HT has been demonstrated (Björklund *et al.*, 1971). Jones (1982b) suggested this indoleamine might be T. A T-containing raphé-hypothalamic pathway has also been suggested (Cox *et al.*, 1981a). Interestingly, a recent study by Broadbent and Greenshaw (1985) indicated differential effects of the 5-HT agonist quipazine and of T on lateral hypothalamic self-stimulation in rats [with no effect and a decrease in response rate, respectively]. However, as the effect of T was only noticed at the highest dose (80 mg kg^{-1}), it was suggested that the reduction in self-stimulation might actually be secondary to a disruption of response performance. Although electrolytic lesions of the raphé nuclei resulted in reductions of approximately 70% and 30% in 5-HT and T brain concentrations, respectively (Knott *et al.*, 1974; Marsden and Curzon, 1974), indicating a possible existence of such pathways, a later

study by Juorio and Greenshaw (1985) demonstrated unaltered hypothalamic T levels following electrolytic lesions of the midbrain raphé nuclei. Intranigral lesions with 5,7-DHT resulted in unaltered striatal and olfactory tubercle T levels (Juorio *et al.*, 1987a).

A number of studies has demonstrated that specific receptors mediating 5-HT-induced contraction in the rat caudal artery appear to be of the 5-HT_2 subtype (Van Neuten *et al.*, 1981; Bradley *et al.*, 1983). Although an earlier report had suggested that an α-adrenoceptor-mediated component was also involved in the isolated rabbit aorta response to T (Stollak and Furchgott, 1983), Bradley *et al.* (1985) later showed that T-induced vasoconstriction in rat artery was predominantly mediated by the 5-HT_2 receptor. The discrepancies between these studies as well as that of Hicks and Langer (1983) were attributed to differences in tissue preparation. Perfused tissue was used by Stollak and Furchgott (1983) and by Hicks and Langer (1983) therefore leaving the question open as to whether an equilibrium between agonist and antagonist was achieved. In contrast, Bradley *et al.* (1985) used a fixed volume bath preparation containing the MAO inhibitor iproniazid. The reason for the inclusion of an MAO inhibitor in their study was that the differential accessibility of T and 5-HT to MAO had previously been shown to account for differences in agonist potencies (Handschumacher and Vane, 1967). A recent study by Frenken and Kaumann (1988) has overruled any possibility of an α-adrenoceptor-mediated response to T-induced contraction in the calf coronary artery. These authors have demonstrated a biphasic effect of T on a 5-HT receptor (specifically the 5-HT_2 receptor), similar to that observed in the neuronal cell firing studies mentioned above, characterized by an early fast contraction followed by a late, slow-developing contraction. This has been attributed to T binding to the 5-HT_2 receptor in a highly active state (R: fast contraction) and in a low active state (R': slow contraction). T also modulates its own and 5-HT-induced effects by binding to an allosteric site which isomerizes the receptor from R to R' (Frenken and Kaumann, 1988). Thus, 5-HT antagonists which compete with T for the allosteric site could potentiate (methysergide; R to R') or attenuate (ketanserin; R' to R) the late T-induced slow contractions

(and *vice versa* for the early fast contractions).

Other transmitter systems: Baker *et al.*(1980b) demonstrated a relatively potent inhibitory effect of T on DA uptake in rat striatal prisms. A similar observation was made using brain tissue slices (Heikkila and Cohen, 1974), although Ennis *et al.* (1981) failed to confirm this effect. It is also known that the behavioural syndromes elicited in animals following T administration can be blocked by DA antagonists (Squires, 1978). The K^+-stimulated release of DA from striatal slices is attenuated by T (Ennis *et al.*, 1981), although a T-enhanced release of DA from non-stimulated striatal prisms has also been observed (Baker *et al.*, 1980b; Baker and Yasensky, 1981). Both intranigral and intraventricular administration of 6-OHDA results in decreased striatal T levels (Marsden and Curzon, 1974; Juorio *et al.*, 1987a), as well as an increase in striatal [^{3}H]-T binding (van Nguyen *et al.*, 1989). The striatum has also been demonstrated as having a substantial [^{3}H]-T binding site density (Cascio and Kellar, 1983; Wood *et al.*, 1984; Altar *et al.*, 1986). van Nguyen *et al.* (1989) have demonstrated the existence of postsynaptic [^{3}H]-T binding sites on DA neurons of the striatum and that these sites are different from DA binding sites. These latter authors found that haloperidol (a D_2 DA antagonist) inhibited neuronal firing induced by apomorphine (a mixed DA agonist) but had no effect on subsequent T-induced firing. The administration of DA antagonists has also been shown to increase striatal T concentrations (Juorio, 1982; Martin *et al.*, 1988), while the opposite effect was observed with (+)-amphetamine (Juorio, 1982). The suggestion that TRP decarboxylase activity, and hence T concentrations, might actually be controlled by the DA system (Juorio and Paterson, 1990) is based on the observation that AAD activity in rat retina and brain is modulated by DA receptors (Hadjiconstantinou *et al.*, 1988, 1989; Rossetti *et al.*, 1990).

Both Cox and Ennis (1982) and Robinson and Marsden (1984) observed an attenuation of the T-induced decrease in 5-HT release following atropine treatment, suggesting an involvement of muscarinic receptors. A slight attenuation by T of cortical neuron responses

to acetylcholine (ACh) substantiates this suggestion (Jones and Boulton, 1981). Physostigmine (an ACh-esterase inhibitor) and scopolamine (a muscarinic antagonist) have opposing actions on certain T-induced behavioural effects (Delini-Stula and Radeke, 1985). Furthermore, a biphasic effect of T on the ACh-mediated frog neuromuscular junction has been observed (Friedman, 1979). Low concentrations of T increase the endplate potential, whereas high concentrations reduce it. Similar effects were observed in the lobster, where glutamate appears to be the active transmitter (Friedman *et al.*, 1981). Krenjvic and Phillis (1963) observed that T antagonized glutamate-induced firing of rat cortical neurons. A strong reduction of substance P-evoked excitation of cortical cells was found following application of T, although no reversal of responses was seen (Jones, 1984; *cf* effects of 5-HT responses of cortical cells). Environmental light increases AAD activity in rat retina, apparently as a result of diminished DA levels (Hadjiconstantinou *et al.*, 1988), although this tissue has been shown to also contain melatonin (Pang *et al.*, 1977). Melatonin, a product of the pineal gland, increases AAD activity, presumably via induction of the AAD co-factor pyridoxal phosphate (Anton-Tay *et al.*, 1970). Orally administered melatonin has also been shown to result in a two-fold increase of lumbar and cisternal IAA concentrations, indicating an increased rate of T turnover (Young *et al.*, 1984). Rhesus monkey plasma cortisol levels increase following *iv* T (Murphy *et al.*, 1985).

It is interesting to note that the effects of T in this last section are related to a variety of other receptor systems (i.e. α-adrenergic, muscarinic and 5-HT_2). It has recently been shown that, as with these other receptors, T receptors stimulate inositol phosphate hydrolysis (Conn and Sanders-Bush, 1985; Osborne *et al.*, 1986; Osborne and Hogben, 1988). However, neither atropine (muscarinic antagonist), phenoxybenzamine (α-antagonist), nor cyproheptadine (serotonergic antagonist) counteracted the stimulation of inositol phosphate metabolism by T (Osborne *et al.*, 1986). Other studies using the 5-HT_2 antagonists methysergide and ketanserin demonstrated a reduction in the metabolism induced by T, suggesting that T might act via the 5-HT_2 receptor subtype (Conn and Sanders-Bush, 1985; Osborne and

Hogben, 1988). It is also quite possible that the true T receptor does not use inositol phosphate as a second messenger, but that T stimulates the metabolism of the latter *in vitro* simply because of its structural similarity to 5-HT (Osborne and Hogben, 1988). A recent report has implicated T in the control of the adenyl cyclase system, following the observation that T blocks the accumulation of cAMP in mouse retina (Cohen and Blazinski, 1987).

2.C.5 Behavioural Effects of Tryptamine

Visible behavioural effects of administered T appear to be excitatory in nature, although exceptions have also been reported. In rats, T excitation develops if an excess of T is present (i.e. *via* exogenous T application) or if both forms of MAO are almost completely inhibited (Foldes and Costa, 1975; Deaken and Green, 1978; Marsden and Curzon, 1978; Irons *et al.*, 1984). Tedeschi *et al.* (1959) observed a series of behaviours, including straub tail, tremor, limb myoclonus and backward locomotion, following *iv* T. These behaviours have come to be described as part of a set of generalized behaviours called the 5-HT syndrome (Jacobs, 1976). Tedeschi *et al.* (1959) further demonstrated potentiation of these behaviours following a MAO inhibitor pretreatment. T-induced, MAO-potentiated clonic effects were also observed in the rabbit (Quock and Weick, 1978) and guinea pig (Luscombe *et al.*, 1982). Homologous behaviours were observed in immature and adult fowl (Dewhurst and Marley, 1965; Marley and Nitisco, 1975; Jones, 1981). T also enhances the TRP-evoked behavioural syndrome (Marsden and Curzon, 1978, 1979; Atterwill and Green, 1980). The exact role of T in the 5-HT syndrome, however, remains unclear. Although the behavioural syndrome induced by combined MAO inhibitor and T treatment only differs from the 5-HT syndrome by the lack of tremors and wet-dog shakes (Marsden and Curzon, 1979), the behaviours elicited by T are attenuated by the 5-HT antagonists methysergide (Dewhurst and Marley, 1965; Luscombe *et al.*, 1982) and metergoline (Gerson and Baldessarini, 1980). This suggested an involvement, albeit indirect, of central 5-HT systems, since it had also been demonstrated that *p*CPA treatment abolished the behaviour induced by combined MAO

inhibitor and T treatment (Marsden and Curzon, 1978; Luscombe *et al.*, 1982; Irons *et al.*, 1984). In addition, treatment with centrally active decarboxylase inhibitors (RO4-4602 and NSD-1055) prevented the 5-HT syndrome and decreased brain T levels but did not affect brain 5-HT levels (Marsden and Curzon, 1979). In contrast, 5-HTP treatment in combination with a peripheral decarboxylase inhibitor elicited the 5-HT syndrome, even though T levels remained unaffected (Modigh, 1972). T enhanced the behavioural syndrome induced by intrathecal administration of 5-HT in mice (Larson and Wilcox, 1984). Although the effects of 5-HT were enhanced by fluoxetine, this compound did not affect the behaviour elicited by combined T and 5-HT treatment, thus suggesting to these authors that T enhanced the effect of 5-HT by competing for a similar uptake site. This prolonged the synaptic availability of 5-HT (Larson and Wilcox, 1984). Delini-Stula and Radeke (1985) stated that T-induced behavioural effects (vocalization, tremor and backward movements) are not dependent on the release of other monoamines since these effects are potentiated by prior reserpine treatment (22 h). While the intensity of the behaviours appears to be associated with brain T content rather than with that of 5-HT (Marsden and Curzon, 1978; Sugimoto *et al.*, 1986), the degree of reduction of any one behaviour, following *p*CPA treatment, is dependent on the amount of 5-HT available (Irons *et al.*, 1984). Following the observation that the specific 5-HT_{1A} agonist 8-OHDPAT induces the 5-HT syndrome in rats and mice (Tricklebank *et al.*, 1984; Yamada *et al.*, 1988), these latter authors proceeded to show that the 5-HT syndrome induced in mice by T is suppressed by the 5-HT_1 antagonists methysergide and (-)-propanolol and the 5-HT_{1A} antagonist spiperone, but remains unaffected by the 5-HT_2 and 5-HT_3 antagonists ketanserin and ICS 205-930, respectively (Yamada *et al.*, 1989), thus leading to the suggestion that T induces its effect via an action at the 5-HT_{1A} receptor. Leysen *et al.* (1978) had shown previously, however, that T-induced seizure activity could be prevented by 5-HT_2 receptor antagonism. The involvement of the 5-HT system is further supported by the observation that T affects both 5-HT uptake and release in the rat (Horn, 1973; Baker *et al.*, 1980b), and that simultaneous *icv* T and 5-HT administration apparently

reduces the dose of 5-HT required to produce the 5-HT syndrome (Dourish and Greenshaw, 1983) and head-twitch (Orikasa, 1988). Jones (1981) observed that T enhanced 5-HTP-induced behavioural symptoms, although it antagonized 5-HTP-induced head-twitches. Dourish and Broadbent (1984) found that, whereas a low dose of T in combination with a low dose of 5-HT reduced food intake, either of these compounds alone, at these doses, was without effect. A high dose of T, however, induced an anorectic effect (Dourish and Broadbent, 1984; Fletcher and Paterson, 1989) which was not affected by prior treatment with *p*CPA, thus leading the latter authors to propose that the anorectic effects of T are unlikely due to an interaction with 5-HT.

Recent evidence indicates a possible DA component in the expression of certain of the T-induced 5-HT syndrome behaviours. Although the neuroleptic chlorpromazine inhibited T-induced behaviours (Tedeschi *et al.*, 1959), this compound has recently been shown to have an equal affinity for both DA and 5-HT receptors (Ortmann *et al.*, 1982). However, the 5-HT_2 receptor antagonist pirenperone which also acts as a potent DA receptor antagonist, has also been shown to inhibit MAO inhibitor/TRP induced head-weaving and abduction of the hindlimb (Green *et al.*, 1983). This is supported by the observation that treatment with haloperidol also reduced the expression of these two behaviours (Yamada *et al.*, 1987a). Furthermore, Jacobs and Klemfuss (1975) and Andrews *et al.* (1982) both demonstrated a reduction in head-weaving in rats following 6-OHDA-induced lesions of the striatum, whereas Delini-Stula and Radeke (1985) observed an inhibition of T-induced behaviours in neuroleptic-treated rats. Tricklebank *et al.* (1984) report that DA receptor antagonists inhibit the 5-HT syndrome induced by the selective 5-HT_{1A} receptor agonist 8-OH-DPAT, whereas manipulations of the DA system also affect the stereotyped responses induced by the 5-HT agonist 5-MeO-DMT (Dickinson and Curzon, 1983). Altar and co-workers (1986) had suggested that T in the nucleus accumbens might interact with DA to modulate locomotor activity, but, Fletcher (1988) could not confirm this as T neither altered the level of

spontaneous locomotor activity nor the locomotor activity induced by systemic administration of (+)-amphetamine. However, Marien *et al.* (1987) noticed that the degree of motor stimulation produced by injection of α,α-[^{2}H]-T into the nucleus accumbens was comparable to that produced by similar applications of (+)-amphetamine or DA (Pijnenburg *et al.*, 1976; Svensson and Ahlenius, 1983). This increase in locomotor activity cannot be attributed to increased synaptic 5-HT levels in the nucleus accumbens because 5-HT injections into this region result in either a transient decrease in locomotor activity or no significant effect (Pijnenburg *et al.*, 1976; Gerber *et al.*, 1986).

Although all of the behavioural effects of T discussed above were excitant, not all studies report such an effect. Ernst *et al.* (1961) and Schain (1961) both observed a decrease in locomotor activity, even to the point of catatonia, following *icv* injections of T in the cat. A similar loss of spontaneous locomotor activity and catatonia were reported in mice and rats, respectively, following T administration (Saavedra *et al.*, 1970). The catatonia observed in the rat was associated with tremor and rigidity, however, thus possibly implying central excitatory effects (Jones, 1982d).

2.C.6 Tryptamine Binding Sites

The concept of a T "receptor" had been alluded to as early as the 1950s (Gaddum, 1953; Wooley and Shaw, 1957) and was later elaborated upon by Dewhurst (Dewhurst, 1968a,b; see Section 2.C.7). It was not until the early 1980s, however, that a specific binding site for T was demonstrated (Kellar and Cascio, 1982; Perry *et al.*, 1982; see Greenshaw and Dewhurst, 1987). The initial study by Kellar and Cascio (1982) found that [^{3}H]-T binding was saturable, with a maximum cortical binding site density (B_{max}) of 429 fmol mg^{-1} protein, and of high affinity, with a dissociation constant (K_D) of 2.8 nM.

Regional and Subcellular Distribution: Studies on the regional distribution of [^{3}H]-T binding sites in rat brain indicate the highest densities in striatal, cortical and hippocampal tissue, whereas the lowest densities are found in the pons, medulla and spinal cord (Cascio

and Kellar, 1983, 1986; Wood *et al.*, 1984). The K_Ds do not vary between regions. *In vitro* autoradiographic quantitation of these binding sites shows a similar distribution pattern with the more rostral structures such as the nucleus accumbens, interpeduncular nucleus, choroid plexus and locus coeruleus, having the greatest densities of [^{3}H]-T binding sites and spinal cord having the least (McCormack *et al.*, 1983; Wood *et al.*, 1985; Altar *et al.*, 1986; Kaulen *et al.*, 1986; Perry, 1986). The hippocampus, which had previously been shown to exhibit moderate [^{3}H]-T binding, actually displays substantial [^{3}H]-T binding in the strata oriens and radiatum, thus making these layers quite important regions in the distribution of [^{3}H]-T binding (Wood *et al.*, 1985; Kaulen *et al.*, 1986). Within each of these two layers, the distribution of [^{3}H]-T binding sites displays a rostral dominance (Kaulen *et al.*, 1986). A site with similar binding parameters has been demonstrated in human cortex (Cascio and Kellar, 1983) and in dog brain where it is regionally expressed in a pattern similar to that seen in the rat (McCormack *et al.*, 1986). Brüning and Rommelspacher (1984) have demonstrated that a similar [^{3}H]-T binding site is also found in peripheral tissues, with densities being high in the liver, intermediate in the heart, and low in other peritoneal organs and the lung. Perry (1986) has found a [^{3}H]-T binding site in the choroid plexus which is pharmacologically distinct from the previously mentioned site. Several compounds, including kynuramine, a potent inhibitor of [^{3}H]-T binding at the brain site (Charlton *et al.*, 1984), failed to affect [^{3}H]-T binding at the choroid plexus site. The possibility that the [^{3}H]-T binding site might in fact be MAO stemmed from two facts. The first was the observation that subcellular fractionation studies disclosed the presence of binding sites primarily in the synaptosomal fraction, although a significant portion of the sites seemed to accumulate in the mitochondrial fraction (Cascio and Kellar, 1983). The second was the observation that the presence of twice the concentration of pargyline (i.e. 20 μM) as that used in standard [^{3}H]-T binding protocols resulted in a 91% decrease in [^{3}H]-T binding site density (Kienzl *et al.*, 1985). Karasawa *et al.* (1986) compared the effects of 10 and 20 μM pargyline in the incubation buffer and found no difference in the amount of [^{3}H]-T binding.

Perry *et al.* (1988) overruled any possibility of MAO-binding by demonstrating that neither competition studies for the [^{3}H]-T binding site and [^{3}H]-labeled MAO nor subcellular and autoradiographic distribution studies of these two sites gave any evidence that [^{3}H]-T is labeling MAO in rat brain. The interaction between T and 5-HT (see Section 2.C.4) suggests that the binding site for T might be a subtype of 5-HT receptor. Kaulen *et al.* (1986) have determined that no correlation exists between the distribution of 5-HT receptors (both 5-HT_1 and 5-HT_2) and [^{3}H]-T binding sites, thus negating any possibility of these sites being the same. This confirms the findings of other studies (Charlton *et al.*, 1984; Perry, 1986). Brüning and Rommelspacher (1985) and Kaulen *et al.* (1986) have both also shown that the [^{3}H]-imipramine binding site differs from that of [^{3}H]-T. van Nguyen and coworkers have demonstrated that although the [^{3}H]-T binding site is different from the DA D_2 site, [^{3}H]-T binds postsynaptically to nigral DA neurons (van Nguyen and Juorio, 1989b; van Nguyen *et al.*, 1989).

Characterization of the Binding Site: Investigations into the inhibitory potency of a variety of ligands, both endogenous and otherwise, revealed three active classes of compounds able to displace [^{3}H]-T binding. These were T analogues, β-carbolines and substituted phenylethylamines, with T displaying the highest affinity for the site (Cascio and Kellar, 1983; Brüning and Rommelspacher, 1984; Wood *et al.*, 1984; Graham and Langer, 1987). Ring and side-chain substitution of the T nucleus resulted in decreased potencies to the point where the N,N-dialkyl-analogue and 5-MeO-N-acetyl-T (melatonin) displayed negligible activity at the site (K_i values of >50,000 nM). Wood *et al.* (1984) further reported on the stereospecificity of the [^{3}H]-T binding site, leading to the suggestion that this binding site might represent the T receptor in brain. β-Phenylethylamine, β-carboline and kynuramine also exhibit nM affinity for this site, this affinity being lost following side-chain substitution of the parent nucleus (Cascio and Kellar, 1983; Wood *et al.*, 1984; Charlton *et al.*, 1984). Unlike T and β-carboline, however, ring substitution of the phenylethylamine nucleus, specifically in the *para*-position, resulted in relatively high

potencies for competition at the [^{3}H]-T binding site (Martin *et al.*, 1984; Graham and Langer, 1987). Perry (1986) and Kaulen *et al.* (1986), using quantitative autoradiography, demonstrated a low affinity of 5-HT and 5-HT receptor anatagonists for the site. This confirmed the findings of other studies using membrane preparations (Cascio and Kellar, 1983; Brüning and Rommelspacher, 1984; Graham and Langer, 1987). None of the investigated hallucinogens, antipsychotics or antidepressants displayed any significant affinity for the site (K_i values of >15 μM), except clorgyline which exhibited much higher affinity (K_i value of 1.98 μM) (Cascio and Kellar, 1982, 1983; Graham and Langer, 1987; Martin *et al.*, 1987; Perry *et al.*, 1988). It is interesting that the (+)-isomers of deprenyl, α-methyltryptamine and amphetamine, all display greater potency than their (-)-counterparts at inhibiting [^{3}H]-T binding (Cascio and Kellar, 1983; Wood *et al.*, 1984; Perry *et al.*, 1988).

[^{3}H]-T binding has also been investigated under a variety of incubation conditions. [^{3}H]-T binds at 0°C with rate constants for association (K_1) and dissociation (K_{-1}) being 0.024 nM^{-1} min^{-1} and 0.041 min^{-1}, respectively, giving a K_D (K_{-1}/K_1) of 1.7 nM. This is in agreement with the K_D estimate achieved using Scatchard analysis of the site in brain and peripheral tissue (Cascio and Kellar, 1983; Brüning and Rommelspacher, 1984). At 37°C, dissociation occurs much more rapidly, thus resulting in much lower specific binding. Karasawa *et al.* (1986) state that the differences in dissociation rates at these two temperatures indicate a temperature-sensitive interconvertable conformation displaying both a high- and low-affinity state of the receptor. This seems unlikely, however, since [^{3}H]-T binding displays a Hill coefficient of near unity, suggesting a single population of binding sites and a lack of cooperativity between the sites (Cascio and Kellar, 1983). The effect of buffer constituents has also been examined. Of the five cations studied to date (Na^+, Ca^{++}, K^+, Li^+, Mg^{++}) only the monovalent ions inhibited binding to any significant degree, and of these only Na^+ affected binding within its physiological range (Cascio and Kellar, 1983). These authors attributed this effect primarily to a reduction in the affinity of the site for [^{3}H]-T. However, clumping of synaptosomes in the presence of metal ions had been noted earlier (Whittaker,

1969; De Feudis and Black, 1973), resulting in a reduction of the physical size of the synaptosomal pellet (Boulton and Baker, 1974). This effect might well account for some of the cation-induced decrease in [^{3}H]-T binding. Karasawa *et al.* (1986) did not find any effect of cations on [^{3}H]-T binding, although the concentration ranges studied in their experiments were low (1 to 10 mM). Neither did these authors observe any effect of ascorbic acid, EGTA or guanine nucleotides when added to the preincubation medium. The addition of glutaraldehyde (0.001 and 0.01%), however, significantly reduced [^{3}H]-T binding (Karasawa *et al.*, 1986; Serikyaku and Ishitani, 1988). This was interpreted as a result of decreased membrane fluidity by protein crosslinking, thus inhibiting the conversion of the [^{3}H]-T binding site from a low- to a high-affinity state. Solubilization studies indicate that the [^{3}H]-T binding molecule is a membrane protein containing a sulfhydryl group (Brüning and Rommelspacher, 1989). Sulfhydryl groups are highly reactive and have been shown, in the case of D_1 DA receptors, to be apparently involved in the maintenance of conformation and biological activity of the site (Dewar and Reader, 1989). The exact molecular mass of the [^{3}H]-T binding site remains questionable, however, due to the lack of sensitivity of the separation technique (Brüning and Rommelspacher, 1985a,b).

Modulation of the Binding Site: The possible role of T in the etiology of psychiatric disorders (see Section 2.C.7) has led many research groups to conduct investigations into the long-term effects of drug administration. Chronic administration of pargyline to rats resulted in a decrease in [^{3}H]-T binding in cortex, striatum, hypothalamus and hippocampus whereas *icv* injection of 6-OHDA and 5,7-DHT did not alter such binding (Cascio and Kellar, 1986). This indicated respectively that a change in T metabolism alters [^{3}H]-T binding and that these binding sites are not located on DA or 5-HT neurons. Although Brüning and Rommelspacher (1985b) found similar results with 5,7-DHT, van Nguyen *et al.* (1989) demonstrated an increase in striatal [^{3}H]-T binding following intranigral 6-OHDA injections, thereby suggesting that a decrease in striatal T (as a result of nigro-striatal neuron loss) was cause for the observed increase in [^{3}H]-T binding and that these binding sites were

post-synaptic in the striatum. Chronic treatment with reserpine (2.5 mg kg^{-1}, 12 d) resulted in increases in [^{3}H]-T binding site densities in the cortex and hippocampus, although binding remained unaffected in striatum and hypothalamus (Cascio and Kellar, 1986), implying possible reserpine-sensitive storage of T in the first two brain regions. Inhibition of 5-HT synthesis with *p*CPA, either acutely or chronically, did not alter [^{3}H]-T binding, although combined long-term treatment (14 d) with clorgyline (1mg kg^{-1} *ip*) and (-)-deprenyl (0.5 mg kg^{-1} *ip*) resulted in a significant decrease in binding (Graham and Langer, 1987). This latter effect was credited to clorgyline, as chronic treatment (1 mg kg^{-1}, 11 d) with this specific MAO-A inhibitor reduced [^{3}H]-T binding, whereas chronic (-)-deprenyl (1 mg kg^{-1}, 11d) did not (Martin *et al.*, 1987). Chronic treatment with the neuroleptic molindone was only effective in reducing [^{3}H]-T binding at a high dose (40 mg kg^{-1} per d). This dose of molindone was shown to have substantial MAO-A-inhibiting activity compared to the lower dose of 5 mg kg^{-1} per d (van Nguyen and Juorio, 1989b). Neither of the tricyclic antidepressants studied to date, i.e. trimipramine and amitryptiline, exert any effect on the [^{3}H]-T binding site (Juorio *et al.*, 1990), and Kellar and Cascio (1985) also failed to find a change in [^{3}H]-T binding site density following either Li^+ (4 wk; serum levels of 0.9 mEq/L), electroconvulsive shock (12 d; once a day) or chronic amphetamine treatment.

2.C.7 Relevance to Psychiatry

Disturbances in the synthesis and metabolism of T have been suggested as being possible factors in the etiology of psychiatric disorders. Dewhurst (1968a,b) put forward an interesting theory regarding three groups of amines which could affect behaviour. Type A amines are non-hydroxylated, very lipid soluble and excitant in nature, and thus can induce exploratory behaviour and vocalization. Examples of Type A amines are PEA and T. Type C amines have a catecholic nucleus, are not very lipophilic and are depressant in nature (i.e. NA and adrenaline). Type B amines are characterized by intermediate chemical and behavioural properties, and are mono-hydroxylated (i.e. *p*- and *m*-TA). Dewhurst (1968a,b)

further suggested that euphoriant substances act on the methysergide-sensitive Type A receptors and has shown that five manic patients given methysergide all improved within days. Significant increases in urinary excretion of T in schizophrenics have been observed to coincide with the manifestation of psychotic episodes (Brune and Himwhich, 1962; Herkert and Keup, 1969). Combined treatment with tranylcypromine and the methyl donor methionine resulted in increased urinary T and an exacerbation of schizophrenic symptoms (Sprince *et al.*, 1963). Rodnight (1961), however, found no difference in urinary T content between schizophrenics and normal controls. Saavedra and Axelrod (1972b) have suggested that N-methylated analogues of T, which were demonstrated in the brain following MAO inhibitor treatment, might well exert a pathogenic effect on brain function (i.e. resulting in psychosis). N,N-Dimethyl-T, for instance, has been identified in psychotic schizophrenic patients being treated with an MAO-inhibiting drug. Normal subjects having received T experience hallucinations (Turner and Merlis, 1959). Urinary T has recently been reported to be altered in the general psychiatric population (Slingsby and Boulton, 1976). Coppen *et al.* (1965) found decreased concentrations of urinary T in depressed patients, although levels of IAA were not correlated with depressed mood. Anderson *et al.* (1984; 1988), studying central T turnover by measuring cerebrospinal fluid IAA levels, found no significant changes in T turnover in subjects suffering from schizophrenia, anorexia, autism, mania or depression when compared to normal controls. Kuehl *et al.* (1968) have indicated certain patients afflicted with Parkinson's disease may excrete abnormally high quantities of T. Although this is in agreement with the findings of Smith and Kellow (1969), other groups have reported normal urinary T levels in Parkinsonism (Brune and Pflughaupt, 1971; Boulton and Marjerrison, 1972). Altered T metabolism has been observed in non-psychiatric conditions such as phenylketonuria (Armstrong and Robinson, 1954; Perry, 1962), pellagra (Sullivan, 1922), thyrotoxicosis (Levine *et al.*, 1962), Hartnup's disease (Baron *et al.*, 1956) and certain cases of carcinoid tumour (Eccleston *et al.*, 1963). Adrenocorticotropic hormone-induced stress might also affect T metabolism (Mandell *et al.*, 1961).

2.D PRODRUGS

The study of an amine's effects on a number of biological parameters may be hampered by its rapid clearance rate or by the necessary use of a MAO-inhibiting drug to help sustain adequate brain levels. This latter approach is flawed, however, in that indiscriminate increases in many amines occur, thus confounding the results. The possibility of delivering the specific amine to its site of action, via a chemical vehicle, is therefore very interesting to the researcher. Such chemical vehicles, or "prodrugs", exist for a variety of compounds. The term "prodrug" or "precursor" was first defined in the late 1950s to describe chemical compounds which undergo biotransformation, or metabolism, prior to eliciting a pharmacological effect (Albert, 1958). The basic premise is that the added chemical moiety is the primary target for enzymatic action, or chemically-mediated reconversion, thus resulting in the liberation of the parent compound under investigation. The terms "latentiated drugs", "bioprecursors", "bioreversible derivatives" and "congeners" are all synonyms of "prodrugs" (Rao, 1987). "Drug latentiation" was described as "the chemical modification of a biologically active compound to form a new compound which upon *in vivo* enzymatic attack will liberate the parent compound. The chemical alteration of the parent compound is such that the changes in physicochemical properties will affect [its] absorption, distribution and enzymatic metabolism" (Harper, 1959). This definition was later revised to include both enzymatic and non-enzymatic reconversion (Kupchan *et al.*, 1965). Traditionally, the specific maneuvering of a drug molecule to its target site involved altering the drug in a manner such that nanoparticles, liposomes, antibodies and synthetic macromolecules acted as media of drug delivery. Drug targeting has been described as being "the ability to achieve a desired pharmacological or other response at a selected site without undesired interaction at other sites" (Rao, 1987). There are two ways of achieving this goal: (a) the vehicular transport approach which involves directing the drug, via liposomes, selectively and specifically to the target site, thus reaching the necessary concentrations for eliciting the

desired response, or (b) the enzymatic or chemical reconversion approach wherein the pharmacologically inert compound is transformed into the active drug at the target site. It is preferable, of course, that the prodrug be inactive *in vivo*. Drug action has been defined as consisting of three levels, none of which is more or less important than another: (a) the pharmaceutical phase, (b) the pharmacokinetic phase and (c) the pharmacodynamic phase (Ariens and Simonis, 1974). Prodrugs are routinely used to overcome barriers that minimize a drug's optimum performance in any of these three phases. A list of these barriers has been compiled by Rao (1987) and is reproduced below:

(a) economics of drug development

(b) esthetic properties for the formulation of the drug and/or dosage, and formulation problems due to the physicochemical properties of the drug in the pharmaceutical phase

(c) problems in the bioavailability of the drug as a result of the drug itself or the dosage, or as a consequence of metabolism (i.e. first-pass metabolism)

(d) transport characteristics of the drug

(e) drug toxicity at the systemic level or at the local level (i.e. irritation at the administration site)

(f) poor site specificity

(g) high absorption and/or elimination rates during the pharmacokinetic and pharmacodynamic phases

As stated above, the main purpose of a prodrug is the delivery of a compound to a specific target site. Although this seems simple enough, there are certain restrictions which dictate whether a prodrug will be successful. Not only must it be accessible to the target site, but the appropriate enzymes, in the case of enzymatic reconversion, must also be readily available. Once it is in the vicinity of the target site, enough of the prodrug must be retained and converted to the parent compound to allow for a pharmacological effect, the latter being solely attributable to the parent compound and not to an unknown effect of the prodrug.

This approach for drug delivery has been employed for a variety of psychotropic agents, including the neuroleptics perphenazine (enanthate ester), fluphenazine (enanthate ester) and penfluridol (an analogue of haloperidol) (Janssen *et al.*, 1970; Van Kempen, 1971; Schreiber and Grozier, 1973), and the tricyclic antidepressant imipramine (pamoate salt) (Wilson *et al.*, 1966). In terms of delivery of specific biogenic amines, the N-ethylcarbamate analogue of PEA and the GABA prodrug progabide were both metabolised *in vivo* to the parent amine (Baker *et al.*, 1984a; Ferrandes *et al.*, 1985).

The N-2-cyanoethyl and the N-methylnicotinic acid amide analogues of T have both been investigated as possible prodrugs of T (Coutts *et al.*, 1985; Bodor *et al.*, 1986; Baker *et al.*, 1987, 1988a). Bodor and colleagues (1986) stated that, although T levels were not sufficiently increased to allow for adequate measurement, changes in DA and 5-HT metabolism following 50 mg kg^{-1} of the amide analogue could be indirect evidence of increased T availability. More accurate investigations were conducted with the N-2-cyanoethyltryptamine (CET) analogue. Following the administration of equimolar doses of T and CET (0.1 mmol kg^{-1}; *ip*), rat brain levels of T were shown initially to be significantly increased after either compound, although CET led to sustained levels of T up to 3 hours post-injection (Coutts *et al.*, 1985; Baker *et al.*, 1987). The latter authors also demonstrated a difference in interactions between T or CET, and other biogenic amine systems. Whereas T resulted in increased DA and 5-HT turnover, CET administration resulted in decreases (Baker *et al.*, 1987). This suggests an inhibitory effect of CET on amine uptake, in contrast to a predominant release effect by T. Rao (1987), investigating MAO activity in CET-treated rats, observed slight, yet selective, MAO-A inhibition *in vitro*, but almost negligible effects *ex vivo*, thus suggesting potential reversible MAO-A inhibiting activity. It is apparent from these reported results that CET elicits neurochemical effects of its own, and is therefore not acting simply as a prodrug.

2.E Aim of the Study

Research into the etiology of affective disorders has concentrated on searching for biochemical abnormalities involving the neurotransmitters NA, DA, and 5-HT and on examining the effects of antidepressant drugs on the uptake, metabolism and receptor activity of these amines (see Sections 2.A-2.B). The involvement of the trace amines in the etiology and pharmacotherapy of affective disorders has been considered by some researchers to be relatively unimportant given the low endogenous levels of these amines. Research into the function of the trace amines has increased following the advent of specific and sensitive assaying techniques and the discovery of specific binding sites for these compounds.

It was the objective of this study to further characterize the binding site for the trace amine T and to investigate the plasticity of this site following chronic antidepressant drug treatments. Analyses, using the rat as an animal model, were undertaken to elucidate the behavioural effects of T on the overall system and to investigate its neurochemical interactions with the classical biogenic amines DA and 5-HT. Behavioural and neurochemical analyses of the N-2-cyanoethyl analogue and, to a lesser extent, the N-propargyl analogue of T were also conducted to examine the potential of these compounds as prodrugs of T.

3 MATERIALS AND METHODS

3.A Analytical Instrumentation and Apparatus

3.A.1 Liquid scintillation spectrometry

A microprocessor-controlled Beckman LS 7500 (Beckman Co., Fullerton, CA, U.S.A.) liquid scintillation spectrometer interfaced with a Datamex Model 43 printer was employed. The LS 7500 is a two or three channel, 300-sample capacity, ambient temperature, soft beta-counting spectrometer. It has the features of automatic quench compensation, H-number quench monitor and 10 library programs with unalterable memory. Two different liquid scintillation cocktails were employed in the scintillation procedures: (a) 0.4 % w/v of butyl-PBD in a mixture of Triton X-100 and toluene (33:66 v/v); (b) Beckman Ready Safe™ liquid scintillation cocktail.

3.A.2 Gas chromatography

Two Hewlett-Packard (HP) gas chromatographs, each equipped with a 15 mCi ^{63}Ni linear electron capture detector (ECD), were employed for the gas chromatographic analyses:

1. A HP 5880A gas chromatograph equipped with a 25 m long fused silica capillary column (0.31 mm I.D., 0.5 μM film of 5% phenylmethyl silicone as stationary phase, Hewlett Packard Co., Palo Alto, CA, U.S.A.). This was interfaced with a HP 5880A Series GC Terminal (level 4) printer/integrator to measure the peak heights. Samples were injected both by hand and automatically using a HP 7672A Automatic Sampler.
2. A HP 5890 gas chromatograph equipped with a 15 m long SE-54 ® fused silica capillary column (0.246 mm I.D., 0.25 μM film of 5% phenylmethyl silicone + 1% vinyl stationary phase, J. & W. Scientific Inc., Rancho Cordova, CA, U.S.A.). This was interfaced with a HP 3392A printer/integrator to measure the peak heights. Samples were injected automatically using a HP 7673A Automatic Sampler.

Helium (2 ml/min) was used as the carrier gas and 5% methane in argon (35 ml/min) was used as a makeup gas on both gas chromatographs. The injector port temperature was 200°C, whereas the oven temperature was programmed to increase as described in the assay method.

3.A.3 High pressure liquid chromatography with electrochemical detection (HPLC-EC)

Brain levels of NA, DA, 5-HT and the acid metabolites DOPAC, HVA and 5-HIAA were determined by reverse-phase HPLC-EC.

HPLC determinations were performed using a Waters (Milford, MA, U.S.A.) WISP 710B sample injector system (set to inject a 15 µl sample), a model 510 pump, and a Bioanalytical Systems (BAS, West Lafayette, IN, U.S.A.) Model LC-4B amperometric detector. A Hewlett-Packard 3392A integrator was used to measure peak heights. The compounds of interest were oxidized at a glassy carbon electrode set against an Ag/AgCl reference electrode set at 0.75 V. The flow rate of the mobile phase was 1 ml/min through an Econosphere-C_{18} column (4.6 mm x 250 mm; 5 µm particle size, Applied Science Labs, Avondale, PA, U.S.A.) coupled to a precolumn composed of the same stationary phase as the analytical column. The mobile phase consisted of $NaH_2PO_4.H_2O$ (55 mM), sodium octyl sulfate (0.85 mM), EDTA (0.37 mM) and acetonitrile (9%). This solution was filtered through a type HA filter (0.45 µm, Millipore) before being degassed and adjusted to a pH of 3.0 using phosphoric acid.

3.A.4 Locomotor activity apparatus

Following the appropriate drug regimen, the locomotor activity of each rat was measured using an activity monitoring system (41.5 cm x 41.5 cm x 30.0 cm, width x depth x height; Acadia Instruments Ltd., Saskatoon, SK, Canada). Locomotor activity was measured as the number of infra-red photobeam interruptions in a 12 x 12 beam floor-grid over the duration of the test period. Two monitoring planes are present in this system, one at 2.5 cm above the floor of the test cage and the other at 15 cm above the floor. The latter 12-beam

array monitored rearing. Beam breaks were divided into three categories and digitally displayed for every 5 min period as:

(a) **total beam breaks;** the total ambulatory activity during the test period.

(b) **consecutive beam breaks;** the number of beam breaks corresponding to the interruption of the same infra-red beam two or more times consecutively. This measure is used as an indication of the occurence of stereotyped behaviours.

(c) **vertical beam breaks;** the number of beam breaks within the upper plane of the activity box. This measure indicates rearing activity.

The number of beam breaks was monitored and stored by an Acadia (modified model 1541) microcomputer. The data were then printed on a Commodore MPS 801 matrix printer.

3.A.5 Glassware

All glassware was allowed to soak overnight in a solution of Sparkleen ® (Fisher Scientific Co., Fairlawn, N.J., U.S.A.) and then sonicated by an ultrasonic cleaner (Baxter/Canlab, Mississauga, ON) for 2 x 45 min in a solution of 2% Decan ® (BDH Chemicals Inc., Toronto, ON) in water. The glassware was then washed in tap water and cleaned in a cold water-hot water-distilled water wash and rinse cycle (Miele dishwasher; Miele Electronics, F.R.G.). Finally the glassware was air dried at 250°-300°C for 1 h in one of two mechanical convection ovens (Model 18EM or Model 28, Precision Scientific Group). In the case of the radioassays, the glassware was soaked for a minimum of two days in Contrad-70 (Baxter/Canlab, Mississauga, ON), rinsed and then sonicated and cleaned, as described above.

3.A.6 Balances

Tissue samples, drugs and chemicals were weighed on a Metler AE160 electronic weighing balance with 0.1 mg sensitivity. Animals were weighed on a 700 series triple beam balance with a 2610 gram capacity (Ohaus, Florham Park, NJ, U.S.A.).

3.A.7 Tissue homogenizer

Tissue samples (brain, liver, heart) for GC analysis and for measurement of monoamine

oxidase activity were homogenized using a Tri-R Stir-R ® homogenizer (Model S63C, Tri-R Instruments, Rockville, NY, U.S.A.) equipped with a Teflon ® pestle and a glass mortar (with a clearance of 0.1-0.15 mm). The rotor shaft has a maximum speed of 12,000 rpm with a ten-speed setting. A speed setting of 7 was routinely used.

3.A.8 Centrifuges

All low-speed centrifugations (up to 1500 g) were performed on a Sorvall GLC-2B benchtop centrifuge (DuPont Instruments). High speed centrifugations (up to 40,000 g) were performed on a Damon IEC B-20A refrigerated centrifuge (Damon/IEC, Needham Hts., MA, U.S.A.). Small-volume high-speed centrifugations were performed on a MSE Micro-Centaur (Baxter/Canlab, Mississauga, ON) benchtop centrifuge.

3.A.9 Shaker-Mixers

A 36-sample IKA-IBRAX-VXR ® (Janke & Kunkel GmBH, Sweden) tube shaker was employed for extraction purposes. Mixing and vortexing of individual tubes was done on a benchtop Thermolyne Maxi Mix 1 ® (Sybron Corporation, Dubuque, IA, U.S.A.). Large volume mixing was done on a benchtop Thermolyne Type 1000, using magnetic stir bars.

3.A.10 Sample filter

Samples for the radioassays were filtered through Whatman GF/B filters via a 24-sample capacity M-24R cell harvester (Brandel, Gaithersburg, MD, U.S.A.). Vacuum for proper filtration rate was maintained with a 760 mm Hg vacuum capacity G8CX benchtop pump (Emerson, St. Louis, MO, U.S.A.).

3.A.11 Ultraviolet Spectrophotometry

The absorbance readings for sample protein content were determined on a Unicam SP1700 Ultraviolet/Visible Spectrophotometer (Pye-Unicam, Cambridge, U.K.) set at a wavelength of 660 nm. A deuterium-tungsten lamp was used as a light source.

3.A.12 pH meter

The pH values of the various buffer solutions were determined by an Accumet model 610

pH meter (Fisher Scientific, Fairlawn, NJ, U.S.A.) standardized using a certified buffer solution (pH 7.00 ± 0.01 @ 25°C; Fisher Scientific).

3.A.13 Sample pipettes

The pipettes used were either preset sample size (10, 25, 50, 100 µl; Socorex, Switzerland), variable sample size (20, 200, 1000 µl capacity; Gilson, France), or repeatable sample (1.25, 5.0, 12.5, 50.0 ml capacity; Eppendorf, U.S.A.) pipettes.

3.A.14 Drug administration

Acute drug administration was accomplished via injection with a tuberculin 1 ml syringe equipped with a 27G1/2 needle (Becton Dickinson, Closter, NJ, U.S.A.). Injections were *via* either intraperitoneal or subcutaneous routes. Chronic drug administration was accomplished using Alzet osmotic pumps models 2002 (2 week duration) and 2ML4 (4 week duration) (ALZA Corp., Palo Alto, CA, U.S.A.). Respective drug solutions were determined using a program written by Greenshaw (1986). The pumps were implanted subcutaneously in the interscapular region of the animal while the latter was under general anaesthetic (diethyl ether). All drug doses are expressed as their salts (see section 3.C). Control animals received the double-distilled water vehicle.

3.A.15 Tissue collection

Animals (see section 3.B) were killed by instant guillotine decapitation. Tissue [whole brain (meninges and pineal gland removed) and liver] was removed as quickly as possible and frozen on solid carbon dioxide. Whenever regional dissection was required, the brain was removed as quickly as possible (maximum 15 sec post-mortum) and immersed in ice-cold saline. The brain was then placed on a saline-soaked filter paper on a bed of ice. Dissections were performed using the protocol described by Juorio *et al.* (1986). The striatum consisted mainly of the head of the caudate nucleus; the hypothalamus was the translucent tissue found below the thalamus between the anterior and posterior commissure; the hippocampus was easily found and dissected out. The frontal cortex was dissected by cutting a coronal section

at the level of the anterior end of the olfactory tubercles and dissecting out the olfactory lobes. All myelin was removed from the tissues and the individual tissues were frozen (all tissues were frozen on dry ice immediately following their removal) and then stored at -80°C until the time of analysis. HPLC and GC analyses of the samples were completed within 3-4 weeks. Radioligand studies were completed within 6 weeks.

3.B Animals

Male Sprague Dawley rats (Bioscience Animal Services, Ellerslie, Alberta, Canada) weighing 250-400 g were used. Weights were not significantly different between groups within individual experiments. All animals were housed in pairs under 12 h light/dark conditions at a room temperature of 21 ± 1°C. Food and water were freely available. Animals were fed Lab-Blox Feed (Wayne Food Division, Continental Grain Co., Chicago, IL, U.S.A.), composed of 4.0% crude fat (min.), 4.5% crude fibre (max.), and 24% crude protein (min.).

3.C Chemicals

The following list comprises all the chemical compounds used throughout this study as well as their suppliers or manufacturers (list continued on next 2 pages).

Chemicals	Suppliers or Manufacturers
Hydroxytryptamine binoxalate, 5-[2-^{14}C] (sp. activity 54 mCi/mmol) Phenylethylamine.HCl, 2-[ethyl-1-^{14}C] (sp. activity 56.8 mCi/mmol) Tryptamine.HCl, [ethyl-^{3}H] (sp. activity 36.7 Ci/mmol) Dihydroalprenolol.HCl, levo-[ring, -propyl-^{3}H(N)] (sp. activity 92.2 Ci/mmol) Imipramine.HCl, [N-methyl-^{3}H] (sp. activity 75.6 Ci/mmol)	New England Nuclear (NEN) (Mississauga, ON, Canada)
N-propargyltryptamine.HCl (PGT) N-2-cyanoethyltryptamine.HCl (CET)	Drs. R.T. Coutts and R.G. Micetich (Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada)

Tryptamine.HCl (T) 2-Phenylethylamine.HCl (PEA) Pargyline.HCl (PARG) (±)-Tranylcypromine.HCl (TCP) Phenelzine sulphate (PLZ) Imipramine.HCl (IMI) Desipramine.HCl (DMI) Apomorphine.HCl (APO) 1-Alprenolol d-tartrate Folin & Ciocalteu's phenol reagent (-)-Noradrenaline.HCl (NA) Dopamine.HCl (DA) 5-Hydroxytryptamine creatinine sulphate (5-HT) 3,4-Dihydroxyphenylacetic acid (DOPAC) Homovanillic acid (HVA) 5-Hydroxyindole-3-acetic acid (5-HIAA) 3,4-Dihydroxybenzylamine (DHBA) 2-(4'-tertbutyl-phenyl)-5-(4"-bi-phenyl)-1,2,3-oxadiazole (butyl-PBD) Di-(2-ethylhexyl)-phosphate (DEHPA) Magnesium chloride ($MgCl_2.6H_20$) 5-Methyltryptamine.HCl (5-MT) Protease (P5380) Bovine serum albumin	Sigma Chemical Co. (St. Louis, MO, U.S.A.)
Clomipramine.HCl (CMI)	CIBA-GEIGY Corporation (Summit, N.J., U.S.A.)
Sodium potassium tartrate	Allen & Hanburys (Toronto, ON, Canada)
Clorgyline.HCl (CLG) (-)-Deprenyl.HCl (DEP)	Research Biochemicals Inc. (Wayland, MA, U.S.A.)
Pentafluoropropionic anhydride (PFPA)	Pierce Chemical Co. (Rockville, IL, U.S.A.)
Octyl sodium sulfate (SOS)	Eastman Kodak Company (Rochester, NY, U.S.A.)
Triton X-100	Terochem Laboratories (Edmonton, AB, Canada)
Chloroform (ACS) Toluene (glass-distilled) Ethyl acetate (glass-distilled) Methanol (HPLC grade) Acetonitrile (HPLC grade)	Caledon Labs (Georgetown, ON, Canada)
Sodium metabisulfite	J.T. Baker & Co. (Philipsburg, NJ, U.S.A.)
Diethyl ether (ACS)	BDH Inc. (Toronto, ON, Canada)

Helium 5% Methane balance argon Nitrogen Air Hydrogen	Union Carbide Canada Ltd. (Edmonton, AB, Canada)
Ammonium hydroxide Sodium carbonate Sodium chloride Sodium borate Sodium hydroxide Sodium phosphate dibasic anhydrous Sodium acid phosphate Potassium bicarbonate Potassium carbonate Potassium chloride Calcium chloride Disodium ethylenediamine tetraacetate (EDTA) Decane Tris(hydroxymethyl)aminomethane.HCl (Tris) L-Ascorbic acid Acetic anhydride Perchloric acid (60%) Hydrochloric acid	Fisher Scientific (Fairlawn, NJ, U.S.A.)

3.D Methodology

3.D.1 [^{3}H]-Tryptamine binding analysis

3.D.1.1 Scatchard analysis (saturation curves)

The assay for [^{3}H]-T binding was adapted from the method of Kellar and Cascio (1982). Cortical tissue was mechanically homogenized in 10 vol of ice-cold 50 mM Tris buffer (washing buffer; pH 7.4 @ 25°C). The homogenates were centrifuged at 40,000xg for 10 min at 4°C. The supernatant was discarded and the resultant pellet was manually homogenized using a Teflon ® pestle and glass mortar (4 up-and-down strokes) as described in section 3.A.7. The homogenate was centrifuged (40,000xg for 10 min @ 4°C), and the final pellet was suspended in 10 vols of 50 mM Tris buffer containing pargyline (10 μM) and ascorbic acid (5.6 mM). This homogenate was pre-incubated at 35-37°C for 40 min in order to inhibit MAO activity (i.e. via pargyline) and to prevent (i.e. via ascorbic acid) the formation

of decomposition products through oxidation (see Table 3).

The saturation experiments were performed in parallel sets (each set in triplicate) of tubes in which a fixed amount of tissue homogenate was incubated with increasing concentrations of the radioligand in the presence (non-specific binding) or absence (total binding) of an excess of unlabeled T (10 μM). Specific binding was defined as the difference between total binding and non-specific binding at each concentration. These tubes were kept on ice for a period of 1 h. Incubation was done in a final volume of 1 ml. The incubation step was terminated by addition of 3 ml of washing buffer followed by rapid filtration through Whatman GF/B filters. The filters were rapidly washed 3 times with 5 ml of ice-cold washing buffer, and were placed into counting vials. Five ml of scintillation cocktail were added to the vials and the latter were allowed to sit overnight before being put into the LS-7500 scintillation counter for determination of radioactive content. Program setting #5 was used for an individual count time of 10 min. Each tube was vortexed at each step to allow for proper mixing of the solutions. A triplicate set of scintillation vials, containing the prefilter amount of radioligand solution, was run for each concentration. This was done to measure total radioactivity added to the corresponding total and non-specific sets of tubes. The EBDA program (Equilibrium Binding Data Analysis; McPherson, 1987) was employed to determine the binding parameters, B_{max} (maximum number of binding sites) and K_D (dissociation constant).

3.D.1.2 Single point determinations

For quick, economical determinations of the effects of drugs on [^{3}H]-T binding, single point binding was used. Tissue aliquots were added to tubes containing a concentration of [^{3}H]-T near the theoretical K_D (approximately 2 nM). The extent of non-specific binding was estimated by the addition of unlabeled T (10 μM) to a parallel set of tubes (see Table 3 for addition volumes). The number of binding sites may be estimated by converting the measured dpm to fmol as shown in the equation :

Tissue	Cortex		Human Platelet	
Binding Site	Tryptamine	DHA	Tryptamine	Imipramine
[^{3}H]Ligand (Specific Activity)	Tryptamine.HCl (36.7 Ci mmol^{-1})	Dihydroalprenolol.HCl (92.2 Ci mmol^{-1})	Tryptamine.HCl (36.7 Ci mmol^{-1})	Imipramine.HCl (75.6 Ci mmol^{-1})
[^{3}H]Ligand Saturation Study	0.5-10.0 nM	0.25-5.0 nM	Single point determination @ 2 nM	Single point determination @ 2 nM
Non-Specific Binding Defined by Drug (10µM)	Tryptamine	1-alprenolol	tryptamine	desipramine (100 µM)
Incubation Buffer	TRIS.HCl (50 mM; pH 7.4) + 5.6 mM ascorbic acid + 10 µM PARG	TRIS.HCl (50 mM; pH 7.4)	Buffers I, II, III, V	Buffers I, II, III, IV
Sample Protein	@450 µg / 100 µl	@600 µg / 100 µl	55 µg sample^{-1}	50 µg sample^{-1}
Incubation Volume	1 ml	1 ml	250 µl	250 µl
Preincubation Temp.	37°C	N/A	37°C	N/A
Preincubation Time	40 min	N/A	40 min	N/A
Incubation Temp.	0-4°C	25°C	0-4°C	25°C
Incubation Time	60 min	20 min	60 min	40 min
Filter Washes With Cold TRIS Buffer (5 ml)	3 times	3 times	3 times	3 times

Table 3: Conditions for radioligand binding assays. Buffer I-V: I; 50 mM TRIS.HCl + 150 mM NaCl + 20 mM EDTA, II; 50 mM TRIS.HCl + 5 mM EDTA, III; 70 mM TRIS.HCl, IV; 50 mM TRIS.HCl + 5 mM KCl + 120 mM NaCl, V; IV + 20 µM PARG + 5.6 mM ascorbic acid.

dpm measured x (1 μCi/2.22 x 10^6 dpm) = X Ci

X Ci x [1 /(spec. act. per fmol ligand)] = Y fmol

It was assumed that the K_D is unchanged following drug treatment. This has been verified in this study by Scatchard analysis (saturation curves on similar drug-treated tissue). Each tube was vortexed at each step to allow for proper mixing of the solutions. An extra set of scintillation vials was run for each concentration so as to measure total added radioactivity.

3.D.1.3 [^{3}H]-tryptamine binding to human platelets

Platelet [^{3}H]-T binding was investigated using a modification of the [^{3}H]-IMI binding assay of Langer *et al.* (1980). Single point binding on outdated human platelets, a donation from the Red Cross Society of Canada (Edmonton, Alta., Canada), was used for this procedure. Platelet-rich plasma (60 ml) was centrifuged at 16,000xg for 10 min (@ 4°C). The pellet was resuspended by vortexing in 20 vol of buffer I , and recentrifuged. The membrane was prepared (i.e. platelet lysis) by resuspension of the pellet in 20 vol of buffer II and manual homogenization with a Teflon ® pestle and glass mortar (3 up-and-down strokes). The resultant mixture was centrifuged at 39,000xg for 10 min (@ 4°C). The pellet was washed by vortexing in 20 vol of buffer III and recentrifuging. This last step was repeated once. The final pellet was resuspended by manual homogenization (3 up-and-down strokes) in 20 vol of buffer IV and preincubated at 37°C for 40 min. The tissue homogenate and appropriate solutions were added to tubes in the amounts listed in Table 3. This mixture was vortexed and incubated at 0°C for 60 min. A portion (100 μl) of the incubated mixture was then diluted with 5 ml of ice-cold buffer IV and quickly filtered through the cell harvester apparatus described in section 3.A.10. The filters were placed in scintillation vials and the radioactivity per vial was determined as described in section 3.D.1.1. An extra set of scintillation vials was run for each concentration to determine total added radioactivity.

3.D.1.4 Characterization of the [^{3}H]-tryptamine binding site

A variety of aspects of the [^{3}H]-T binding site were investigated.

(a) The effect of buffer osmolarity was investigated by altering the Tris content in the buffer solution. Three buffer concentrations (12.5, 25.0 and 50 mM Tris) were used. Single point binding (see section 3.D.1.2) was used to determine the extent of specific binding under each condition.

(b) The effects of cations (Na^+, Ca^{++}, K^+, Mg^{++}) in the incubation buffer were investigated. A range of concentrations (ie. 0.3 to 1,000 mM) of a cation, or combinations of the specified cations, were introduced into the incubation buffer and [^{3}H]-T binding was measured using single point binding. A set of tubes containing a similar range of concentrations of glucose were analyzed simultaneously to account for osmolarity effects over the experimental range.

(c) The nature of the [^{3}H]-T binding site was investigated by the addition of a proteolytic incubation step in the binding assay. Protease (P-5380), a non-selective proteolytic enzyme, was added to the incubation buffer (0.2 mg/ml). A 12 h incubation period (using standard 37°C incubation temperature), to allow for maximal proteolysis, was used. A parallel set of tubes (both control and proteolytic conditions) was incubated at 4°C to compare effects of storage temperature on [^{3}H]-T binding. Single point binding was employed to determine the extent of specific [^{3}H]-T binding.

3.D.2 [^{3}H]-Dihydroalprenolol ([^{3}H]-DHA) analysis

3.D.2.1 Scatchard analysis (saturation curves)

[^{3}H]-DHA binding was used as an added validation method for drug treatment (a feature common to many antidepressants is their ability to decrease [^{3}H]-DHA binding, see Section 2.A). The assay for [^{3}H]-DHA binding was adapted from the method of Bylund and Snyder (1976). DHA is a ligand for β-adrenergic receptors. Cortical tissue was homogenized

mechanically in 10 vol of ice-cold 50 mM Tris buffer (washing buffer). The buffer pH was adjusted to 7.4 (@ 25°C) using 12 N HCl. The homogenates were centrifuged at 40,000xg for 10 min at 4°C. The supernatant was discarded and the resultant pellet was manually homogenized using a Teflon ® pestle and glass mortar (4 up-and-down strokes) as described in section 3.A.7. The homogenate was centrifuged (40,000xg for 10 min @ 4°C), and the final pellet was suspended in 10 vols of 50 mM Tris buffer (see Table 3).

The saturation experiments were performed in parallel sets of tubes in which a fixed amount of tissue homogenate was incubated with increasing concentrations of the radioligand in the presence (non-specific binding) or absence (total binding) of an excess of unlabeled 1-alprenolol (10 μM) (see Table 3). Specific binding was defined as the difference between total binding and non-specific binding at each concentration. These tubes were kept at room temperature for a period of 20 min. Incubation was done in a final volume of 1 ml. The incubation step was terminated by addition of 3 ml of washing buffer followed by rapid filtration through Whatman GF/B filters. The filters were rapidly washed 3 times with 5 ml of ice-cold washing buffer, and were placed into counting vials. Five ml of scintillation cocktail were added to the vials and the latter were allowed to sit overnight before being put into the LS-7500 scintillation counter for determination of radioactive content. Program setting #5 was used for an individual count time of 10 min. Each tube was vortexed at each step to allow for proper mixing of the solutions. A set of scintillation vials, containing the prefilter amount of radioligand solution, was run for each concentration. This was done to measure total radioactivity added to the corresponding total and non-specific sets of tubes. Scatchard analyses for [^{3}H]-DHA binding parameters were conducted in collaboration with D.J. McManus.

3.D.2.2 Single point determinations

The method for single point [^{3}H]-DHA binding was based on the same approach described for single point [^{3}H]-T binding (see section 3.D.1.2). The binding was conducted near the

K_D for DHA (ie. approximately 1.5 nM).

It was assumed that the K_D is unchanged following drug treatment. This has been verified in this study by Scatchard analysis (saturation curves on similar drug treated tissue). Each tube was vortexed at each step to allow for proper mixing of the solutions. An extra set of scintillation vials was run for each concentration so as to measure total added radioactivity.

3.D.3 [^{3}H]-Imipramine ([^{3}H]-IMI) binding to human platelets

Platelet [^{3}H]-IMI binding parameters were also determined on outdated human platelets. The method used was similar to that described in section 3.D.1.3 for [^{3}H]-T binding in platelets, except that 20 vol of buffer V (instead of buffer IV which was used for the platelet [^{3}H]-T binding; see Table 3) were used for the final homogenization step in this assay. The extent of non-specific binding was estimated by the addition of unlabeled desipramine (10 µM) to a parallel set of tubes. Total added radioactivity was determined as described above.

3.D.4 Protein determination

The protein content per sample volume was determined using the method described by Lowry *et al.* (1951). Knowledge of the protein content is a requisite for comparison of individual binding parameters between samples. A set of tubes containing known amounts of bovine serum albumin were run in parallel with the set of samples, thus giving a standard curve for extrapolation. Water was added to each tube in the standard curve to standardize the volume. Volumes of tissue homogenate, similar to those used in the binding studies, were added to individual tubes. Tubes were run in duplicate. Membrane digestor (1:1 1N NaOH:Na deoxycholate; 250 µl) was added and allowed to sit for 10 min. Reagent A (1:1:100 1%$CuSO_4$:2% Na-K-Tartrate:2% Na_2CO_3; 5 ml) was then added and the tubes were left for 10 min. Finally, Folin reagent (1:1 2N Folin-Phenol:dH_2O; 500 µl) was added and left for 30 min. The absorbance of each sample was then read at a wavelength of 660 nm on the spectrophotometer described in section 3.A.11. The Lowry program (McPherson, 1987) was used to determine the protein content per tube. Each tube was vortexed at each

step to allow for proper mixing of the solutions.

3.D.5 Gas chromatographic (GC) analysis of tryptamine by derivatization with pentafluoropropionic anhydride

Whole brain and liver T levels were determined using GC with electron capture detection. The tissue was homogenized in 5 vol of ice-cold 0.1 N $HClO_4$. If monoamine oxidase activity was to be determined (see Section 3.D.8), then the tissue was homogenized in 5 vol of ice-cold distilled H_2O and an aliquot (200 µl) was frozen until the time of assay. The remaining homogenate would have 1/10 the final volume of 1.0 N $HClO_4$ added to it to give a final concentration of 0.1 N $HClO_4$.

A portion (3 ml) of the $HClO_4$ homogenate was added to a tube containing 250 ng of 5-MT (the I.S.). The homogenate in the tube was adjusted to a pH value of 7.8 using solid $KHCO_3$. The tube was vortexed briefly and centrifuged (up to 1,500xg). Sodium phosphate buffer (300 µl, ph 7.8) was added, followed by 5 ml of DEHPA in $CHCl_3$. The sample was then shaken for 4 min and centrifuged at 2,500xg for 10 min. The aqueous layer was discarded and the organic layer was retained and transferred to another tube. HCl acid (0.5 M, 2.5 ml) was added, and the solution was mixed for 4 min and centrifuged at 1,500xg for 5 min. The aqueous layer was removed to another tube and neutralized by addition of solid $NaHCO_3$. Acetylation was accomplished by adding 300 µl of acetic anhydride (Martin and Baker, 1977). After acetylation was complete, ethyl acetate (4 ml) was added and the mixture was shaken for 4 min and centrifuged at 1,500xg for 5 min. The organic layer was transferred to another tube, mixed with 800 µl of distilled H_2O for 10 sec and centrifuged up to 1,500xg. The organic layer was transferred to a set of smaller tubes and taken to dryness under a stream of nitrogen. The resultant residue was reacted with 25 µl of ethyl acetate and 75 µl of pentafluoropropionic anhydride for 30 min at 60°C. The mixture was partitioned between cyclohexane (300 µl) and saturated sodium borate solution (3 ml) by vortexing for 15 sec and centrifuging briefly. An aliquot (1 µl) of the organic layer was injected onto the gas

chromatograph. The initial oven temperature was 80°C (for 30 sec post-injection). The temperature was then increased at a rate of 20°C/min to a maximum of 250°C where it remained for 10 min. The retention time for derivatized T was 10.17 min while the retention time for the I.S. 5-MT was 10.86 min.

The structural similarity between T and 5-HT would allow for simultaneous determination of whole brain 5-HT levels using this procedure (Baker *et al.*, 1980c,1981). The retention time for derivatized 5-HT was 13.49 min. Whole brain levels of T and 5-HT in tissue samples were determined by the peak height ratios of the analytes to the internal standard (IS) in each sample. These ratios were then used in linear regression equations based on the peak height ratios of known amounts of the analyte to a fixed amount of the IS, thus giving a standard curve. A standard curve prepared from a series of concentrations of authentic standards was included in each assay run.

3.D.6 Determination of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA by HPLC-EC

Separate brain regions were homogenized in 300 or 1000 µl (depending on the concentration of the neurotransmitters and their metabolites in the region) of a solution containing $NaH_2PO_4.H_2O$ (55 mM), sodium octyl sulfate (0.85 mM), 0.37 mM EDTA, and acetonitrile (9%). The homogenate was centrifuged at 12,000xg for 15 min (@ 4°C). Aliquots (15 µl) of each supernatant were injected onto the HPLC-EC system described in section 3.A.3. Levels of the neurotransmitter amines and their acid metabolites in tissue samples were determined by the peak height ratios of the analytes to the internal standard (IS) in each sample. These ratios were then used in linear regression equations based on the peak height ratios of known amounts of the authentic analyte to a fixed amount of the IS, thus giving a standard curve. A standard curve consisting of varying concentrations of authentic standards was included in each assay run.

3.D.7 Determination of monoamine oxidase (MAO) activity

Activity of MAO in brain and liver was determined using a modification of the radiochemical procedure of Wurtman and Axelrod (1963) with 5-hydroxytryptamine (5-HT) and 2-phenylethylamine (PEA) as substrates for the MAO type-A and type-B isozymes, respectively. The tissues were homogenized in 5 vols of distilled H_2O and aliquots (200 µl) were diluted to 1000 µl with ice-cold isotonic KCl. Sodium phosphate buffer (250 µl, pH 7.4) and 25 µl of tissue homogenate (25 µl of isotonic KCl in the case of blanks) were added to tubes kept on ice. Aliquots (25 µl) of [^{14}C]-5-HT or [^{14}C]-PEA solutions, suitably diluted with the respective unlabeled compound, were added to each tube to give a final concentration of 100 µM of substrate in each. The tubes were incubated at 37°C for 20 min. The enzyme reaction was terminated with the addition of 200 µl of 2 N HCl. The acid metabolites formed were extracted into 6 ml of toluene by mixing for 3 min and centrifuging at 1000xg for 5 min. The tubes were then kept at -80°C for not less than 1 h to freeze the bottom (aqueous) layer. The toluene layer was transferred to scintillation vials containing 9 ml of scintillation cocktail. Radioactive content per tube was determined by using the liquid scintillation spectrophotometer described in section 2.A.1. The amount of radioactivity in blank tubes was subtracted from all samples and controls. The percentage of MAO inhibition was determined by the equation;

$$\% \text{ inhibition} = 100 - [100 \times ((\text{sample} - \text{blk})/(\text{ctrl} - \text{blk}))]$$

with 'sample' being the radioactivity content per sample, 'blk' being the mean of the blanks, and 'ctrl' being the mean of the control samples.

3.D.8 Synthesis of prodrugs

A brief description of the synthetic methods for N-2-cyanoethyltryptamine.HCl (CET) and N-propargyltryptamine.HCl (PGT) (Coutts *et al.*, 1986) is given below.

3.D.8.1 Preparation of N-2-cyanoethyltryptamine.HCl

A solution of T and acrylonitrile, in a mixture of methanol, water and dimethylformamide

(20 ml) (4:4:1), was heated in an oil bath (80-85°C) for 18 h. After cooling to room temperature, the mixture was concentrated under reduced pressure and the residue was dissolved in water and extracted into chloroform. The organic extract was washed four times with water, then with brine and dried using Na_2SO_4. The crude product was separated by silica gel chromatography using ethyl acetate as the eluant, and gave CET as a pale yellow viscous oil. The free base was converted to its hydrochloride salt and purified by recrystallization from chloroform-ether-methanol.

3.D.8.2 Preparation of N-propargyltryptamine.HCl

A solution of propargyl mesylate in dry tetrahydrofuran (THF) was added dropwise, at room temperature, to a stirred solution of T in dry THF. After 16 hr, the precipitate salt was filtered and washed with ether. The filtrate and washing were concentrated and the residue was dissolved in ethyl acetate. The ethyl acetate solution was washed with aqueous K_2CO_3, then with water and brine, and dried using Na_2SO_4. After filtration, the filtrate was evaporated and separated by silica gel column chromatography using ethyl acetate as eluant. The title compound was isolated, converted to its hydrochloride salt and purified by recrystallization from chloroform-ether-methanol.

Both compounds (sections 3.D.8.1-2) were characterized and authenticated by proton nuclear magnetic resonance, infrared spectroscopy and high resolution mass spectrometry.

3.D.9 Locomotor activity measurements

Locomotor activity was monitored using the apparatus described in section 3.A.4. Animals received their drug regimen and were then monitored for the duration of the test period. Rats are nocturnal animals and thus quite sensitive to light, necessitating that locomotor activity testing was conducted in a room kept at low-intensity light. The test room was separate from other rooms so as to avoid startling or excessive noise. At the end of the test period, the rats were either returned to their respective housing-cages to complete their drug regimen, or they were killed for tissue removal as described in section 3.A.14.

3.D.10 Statistical analyses

Data were analyzed by either one-, two- or three-way analysis of variance (ANOVA) followed by independent t-tests in the case of single pair comparisons and by the Newman-Keuls test in the case of multiple mean comparisons. All probabilities are two-tailed. A probability value $p<0.05$ (i.e., $\alpha=0.05$) was used to establish statistical significance. Statistical significance of behavioural data was explored in one of two manners. In the case of single point binding determinations sample tissue from individual animals was assayed in triplicate (minimum). Binding results are based on a minimum six animals per treatment group, whereas behavioural results are based on a minimum of eight animals per treatment group. In the case where animals underwent behavioural testing on two consecutive days, the results of the second day were expressed as percentages of the first day's respective results. The percentages were then "arcsine" transformed and the appropriate ANOVA was conducted. In the case of a single test period, the results were "square-root" transformed before the ANOVA was conducted. The transformation of behavioural data is an accepted procedure for reducing the error caused by data points not normally distributed (Winer, 1971). The "arcsine" and "square-root" transformed data are presented as percentages and raw data, respectively, for clarity. Statistical analysis on neurochemical data was conducted using the raw data. All statistical analyses were performed on an Epsilon system 1600 personal computer (XT-clone, MS-DOS compatible) using statistical programs written by Mrs. Jordyce van Muyden and Dr. A.J. Greenshaw.

4 RESULTS AND DISCUSSION

4.A Characterization of the [^{3}H]-T binding site

4.A.1 Analysis of [^{3}H]-T binding parameters under control conditions

Introduction: Specific binding sites for T were first discussed in 1982 by Kellar and Cascio (1982) and Perry *et al.* (1982). Research in this area has attempted to properly characterize this binding site with respect to preincubation conditions (cations, ascorbic acid, EGTA, guanine nucleotides and gluteraldehyde), temperature-dependence (Cascio and Kellar, 1983; Karasawa *et al.*, 1986), and subcellular (Perry *et al.*, 1988) and regional (Cascio and Kellar, 1983; Brüning and Rommelspacher, 1984; Wood *et al.*, 1984) distribution. Effects of drug competition (Kellar and Cascio, 1982; Graham and Langer, 1987), lesions (Cascio and Kellar, 1986; van Nguyen *et al.*, 1989) and drug administration, both acutely and chronically (Cascio and Kellar, 1986; Graham and Langer, 1987; Martin *et al.*, 1987; van Nguyen and Juorio, 1989a,b) have also been investigated; however, certain aspects of this site and its binding parameters remain to be fully elucidated. The effects of buffer osmolarity, buffer constituents, temperature, proteolytic conditions, and age of rats on [^{3}H]-T binding were investigated. Preliminary assays were performed to ensure that the parameters were compatible with the [^{3}H]-T site in the literature.

Results: Using the protocol described in section 3.D.1.1, the control values for the B_{max} (the maximum number of binding sites) and the K_D (the dissociation constant) were 210-330 fmol/mg protein and 2.3-5.6 nM, respectively (for example Scatchard transformation see Figure 6). These results are in accord with results describing [^{3}H]-T binding sites in the literature (Kellar and Cascio, 1982: Brüning and Rommelspacher, 1984: Wood *et al.*, 1984; van Nguyen *et al.*, 1989).

The single point binding procedure used for most of the following investigations is described in section 3.D.1.2. The particular assays are described in section 3.D.1.4.

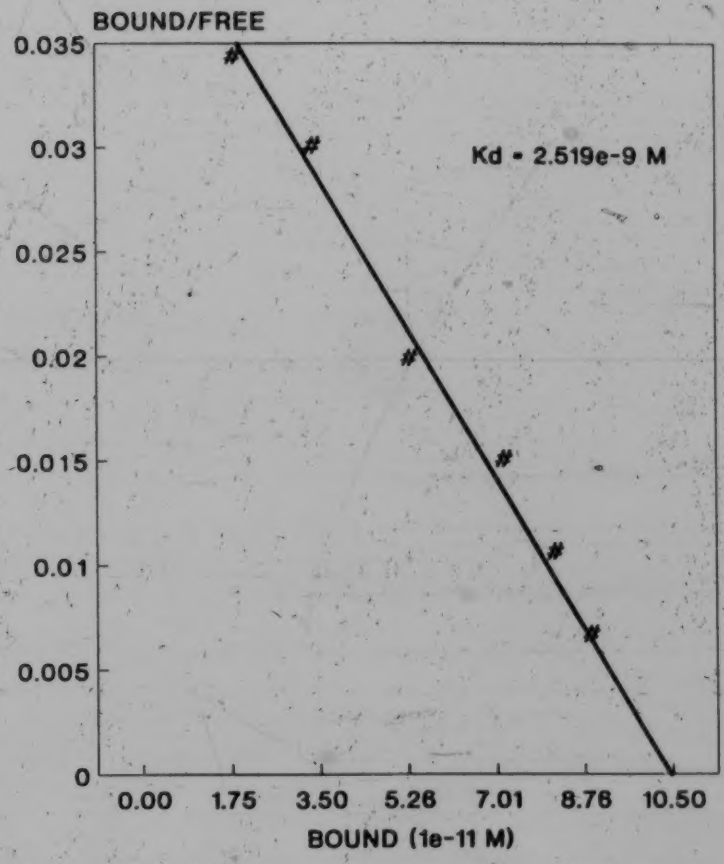


Figure 6: Scatchard transformation for [^{3}H]-T binding under control conditions.

4.A.2 Effects of buffer osmolarity on [^{3}H]-T binding

Varying the Tris buffer osmolarity had profound effects on [^{3}H]-T binding. The 50 mM Tris buffer, being the Tris concentration commonly used, gave significantly higher specific binding than the 12.5 and 25.0 mM Tris buffers [F(2,6)=217.45] whereas non-specific binding was significantly decreased with 50 mM Tris [F(2,6)=16.55] (Table 4).

4.A.3 Effects of cations on [^{3}H]-T binding

To control for any osmolarity effects that addition of cations to the incubation buffer might have, a range of glucose concentrations were investigated. Glucose had no effect on [^{3}H]-T binding across the designated range (0.3-1000 mM).

A similar concentration range of physiologically relevant mono- and divalent cations (Na^+, K^+, Mg^{++} and Ca^{++}) were investigated. The IC_{50} values (concentrations required to inhibit 50 % of specific binding) of these cations with regard to [^{3}H]-T binding varied (Table 5). The monovalent ions were more potent at inhibiting [^{3}H]-T binding than the divalent ions: For Na^+ the IC_{50} was 35.1±5.9 mM, whereas for K^+ the value was slightly lower at 6.1±0.1 mM. Ca^{++} had an IC_{50} of 58.2±0.9 mM whereas >300 mM of Mg^{++} was required to partially inhibit [^{3}H]-T binding. The effect of Na^+ on [^{3}H]-T binding was again observed in a subsequent experiment. In comparison to a control solution containing four cations (Na^+, K^+, Ca^{++}, Mg^{++}: in concentrations consistent with artificial CSF) and glucose (bringing up the total osmolarity to 0.32 M), only a solution lacking Na^+ resulted in an increase in specific [^{3}H]-T binding [F(4,10)=154.56]. In this latter case non-specific binding was also significantly increased [F(4,10)=290.23] (Table 6).

4.A.4 Effects of storage temperature and proteolysis on [^{3}H]-T binding

Specific [^{3}H]-T binding was dependent on the storage temperature and the protein content of the tissue homogenate. The [^{3}H]-T binding protocol calls for an incubation period of 1 hr: However, tissue homogenates in the present experiment were "incubated" at the various

Tris (mM)	Total Binding	Specific Binding
12.5	2035 (30)	331 (18)
25.0	2182 (28)	300 (18)
50.0	3478 (64)*	1900 (105)*

Table 4: Effects of Tris buffer osmolarity on [^{3}H]-T binding to rat cortical tissue. Single point binding was determined at 2 nM [^{3}H]-T. Mean dpm per mg protein ± (SEM); *: significantly different from other buffer conditions.

Buffer Constituent	IC_{50} (mM)
Glucose	no effect
Na^+	35
K^+	6
Ca^{++}	58
Mg^{++}	>300

Table 5: Effects of glucose, Na^+, K^+, Ca^{++} and Mg^{++} (each at 0.3-1000 mM) on [^{3}H]-T binding to rat cortical tissue. Single point binding was determined at 2 nM [^{3}H]-T.

Ion Solution (A)	Total Binding	Specific Binding
(A)	2687 (20)	1338 (52)
(A) - Na^+	4979 (18)*	2516 (26)*
(A) - K^+	2462 (69)	1139 (78)
(A) - Mg^{++}	2329 (29)	1102 (2)
(A) - Ca^{++}	2566 (44)	1201 (47)

Table 6: Effect of buffered ion solution (A), containing Na^+ (125 mM), K^+ (2.63 mM), Mg^{++} (0.91 mM) and Ca^{++} (1.30 mM) in concentrations consistent with Brain Ringer's solution and the effect of the exclusion of either of these cations [(A)-X^n] on [^{3}H]-T binding to rat cortical tissue. Single point binding was determined at 2 nM [^{3}H]-T. Mean dpm per mg protein ± (SEM); *: significantly different from control (A) levels.

temperatures for 12 hr to allow for maximal proteolysis. It was therefore deemed important to differentiate between the standard "incubation" period and the "storage" period used for this experiment, hence the use of the latter term. A significant decrease in specific binding was observed following an increase in the storage temperature (i.e. from 4°C to 37°C) [F(1,15)=154.14]. A similar decrease in specific binding was observed following the addition of a proteolytic enzyme (protease) to the incubation mixture, regardless of the storage temperature [F(1,15)=156.70] (Figure 7). [^{3}H]-T binding must be conducted on the same day as the tissue homogenate is prepared, as freezing (-80°C) the homogenate significantly reduces specific binding [F(1,7)=184.48] (Table 7).

4.A.5 Effect of age on [^{3}H]-T and [^{3}H]-DHA binding and MAO activity

In comparing the [^{3}H]-T binding parameters, B_{max} and K_D (using Scatchard analysis, see section 3.D.1.1), between cortical tissue from mature (3 mth) and old (18 mth) rat, a significant increase in the B_{max} was observed with age (326.3 ± 13.8 fmol/mg protein compared to a control level of 221.7 ± 21.1 fmol/mg protein) [F(1,15)=18.06] (Figure 8). As an added validation method, [^{3}H]-DHA binding parameters were measured (see section 3.D.2.2). The [^{3}H]-DHA B_{max} [F(1,14)=0.77], as well as the K_Ds for both [^{3}H]-T and [^{3}H]-DHA binding, remained unchanged with age.

MAO-A activity decreased significantly with age in whole brain (less cortex) and liver tissue [F(1,18)=7.69 and F(1,18)=9.03, respectively] (Table 8). MAO-B activity remained unchanged, in both types of tissue [F(1,18)=2.04 and F(1,18)=0.65, for brain and liver, respectively], with age.

Discussion: [^{3}H]-T binding is dependent on the osmolarity of the buffer as well as on buffer constituents (monovalent cations have a much greater effect on an equimolar basis than do the divalent cations tested). Increasing storage temperature of the tissue homogenate significantly decreases [^{3}H]-T binding to the protein binding site found in rat brain tissue.

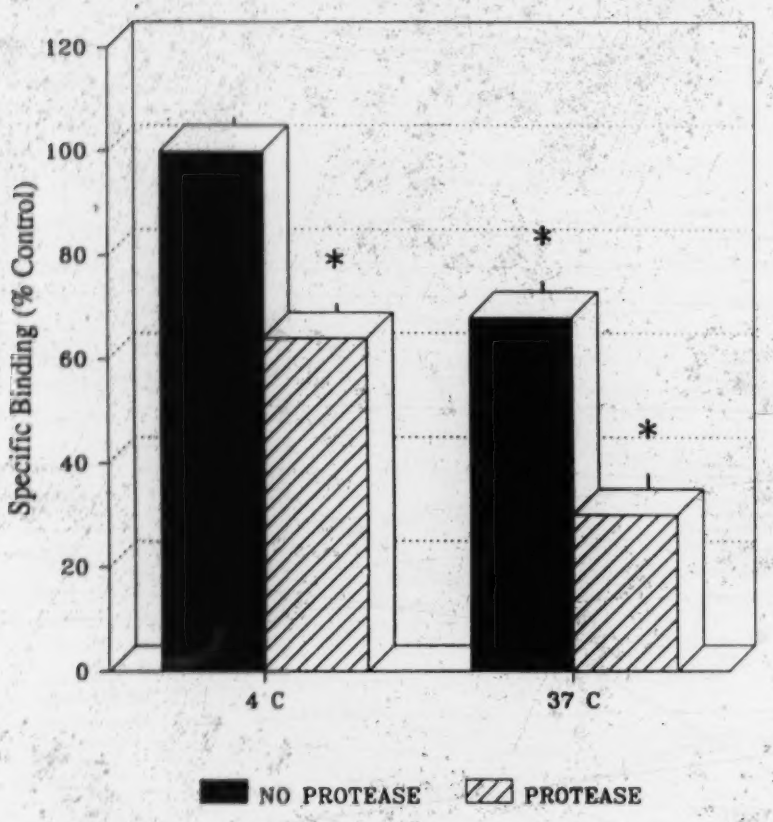


Figure 7: [^{3}H]-T binding site density changes in rat cortex as a result of protein degradation and storage temperature. *: significantly different from 4°C control conditions.

Temperature	Total Binding	Specific Binding
4°C	100 (1)	54 (2)
-80°C	64 (1)*	26 (1)*

Table 7: Effect of tissue homogenate storage temperature on [^{3}H]-T binding to rat cortical tissue. Single point binding was determined at 2 nM [^{3}H]-T and expressed as a percentage of binding at 4°C (control conditions). *: significantly different from control binding.

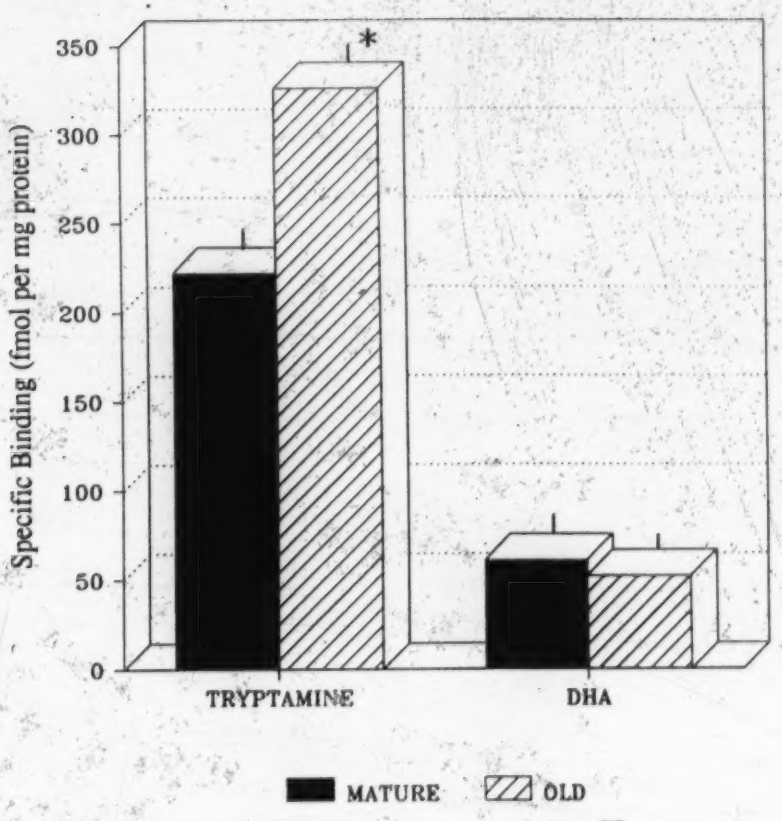


Figure 8: [^{3}H]-T and [^{3}H]-DHA binding site densities in cortex of mature (3 month) and old (18 month) rats. *: significantly different from mature rat ([^{3}H]-T: 222 ± 21 fmol mg^{-1} protein; [^{3}H]-DHA: 61 ± 5 fmol mg^{-1} protein).

Tissue	Age	MAO Activity (nmol/mg protein min^{-1}) MAO-A	MAO-B
Brain			
	Mature	0.310 (0.01)	0.225 (0.02)
	Old	0.262 (0.01)*	0.239 (0.00)
Liver			
	Mature	0.516 (0.02)	0.263 (0.01)
	Old	0.447 (0.02)*	0.280 (0.01)

Table 8: Effect of age on whole brain (less cortex) [top] and liver [bottom] MAO activity in the mature (3 mth) and old (18 mth) rat. Mean ± (SEM); *: significantly different from mature rat tissue MAO activity.

The tests conducted by Cascio and Kellar (1983) with respect to effects of ions on [^{3}H]-T binding revealed that, of the ions tested, only the monovalent ions, and more specifically Na^+ within its physiological concentration range, inhibited specific binding significantly. It is interesting to note that Boulton and Baker (1974), in a study of the subcellular distribution of T, observed a decrease in synaptosomal and mitochondrial fraction T accumulation following addition of Na^+ (5 and 100 mM) to the incubation conditions. The findings in the present study support the findings published by Cascio and Kellar (1983) although differences are obvious between these two studies. The IC_{50} values for the monovalent ions are similar: However, the IC_{50} values for the divalent cations differ. In the study by Cascio and Kellar (1983) an IC_{50} of >150 mM was observed for Ca^{++}, whereas the present study revealed an IC_{50} of approximately 58 mM. Both studies showed little effect of Mg^{++} on [^{3}H]-T binding with Cascio and Kellar (1983) finding an IC_{50} of >100 mM while this study found >300 mM. Kurawasa *et al.* (1986), investigating the effects of a variety of agents on [^{3}H]-T binding, found no effect of cations (Na^+, Ca^{++}, Mg^{++}), but the concentration range investigated was too low and contained only three determination points (1 to 10 mM).

The observation that [^{3}H]-T binding is altered following freezing (-80°C) of the homogenized tissue preparation is of importance when considering storage of the sample(s) and assay procedure. Faull *et al.* (1988) have shown that muscarinic cholinergic, α_1-adrenergic, D_2 DA and 5-HT_2 binding-site densities do not change when newly dissected tissue, without any preparation, is frozen (-80°C). According to these authors binding parameters for the four receptor sites remain unchanged, even if frozen for up to 44 months. It is important to use either fresh or unprepared frozen tissue when conducting [^{3}H]-T binding assays, preferably preparing the tissue on the day of the binding assay.

Ageing, an important physiological factor, resulted in a significant increase in [^{3}H]-T binding in rat cortex, whereas β-adrenergic binding was shown to remain unchanged with age. β-Adrenoceptor density has been shown to be decreased in the brain of aged rats (Scarpace

and Abrass, 1988 for review). However, more specific regional binding studies have demonstrated unaltered β-adrenoceptor densities in cortical membranes from Fischer (Miller and Zahniser, 1988), Sprague-Dawley (Pittman *et al.*, 1980) and Wistar rats (Maggi *et al.*, 1979). An increase in [^{3}H]-T binding should result from a decreased availability of endogenous T according to the principle of denervation supersensitivity and the principle of receptor regulation which states that receptor number is negatively correlated to the availability of the endogenous ligand (Cannon and Rosenblueth, 1949; Creese and Sibley, 1981). The finding in the present study that MAO-A activity decreases, theoretically leading to an increase in T levels, in brain tissue of old rats is therefore in contrast to this principle. The obvious explanation for this discrepancy is that MAO-A (and -B) activity is heterogeneous throughout brain tissue (Petkov *et al.*, 1987; Arai and Kinemuchi, 1988). The finding that MAO-A activity decreased in the "rest of brain" (i.e.brain less cortical tissue) does not necessarily reflect what occurred in the cortical tissue used in the present [^{3}H]-T binding assay. Although Arai and Kinemuchi (1988) found a decrease in MAO-A activity in aged (100 week old) rats, their study was conducted using forebrain homogenates and thus did not indicate what occurs specifically in cortical preparations from aged rats. Indeed, Petkov *et al.* (1987) state that MAO-A activity actually increases in cortical tissue from aged rats. Levels of another trace amine, octopamine, decrease with age (David *et al.*, 1989), presumably as a result of the increase in MAO activity. In addition, the binding of [^{3}H]-imipramine (a ligand for the uptake site of another MAO-A substrate, 5-HT) increases with age (Severson *et al.*, 1985; Marcusson *et al.*, 1987). These results, taken together, are more in keeping with the present finding that cortical [^{3}H]-T binding in aged rats increases, presumably as a result of increased cortical MAO-A activity and, consequently, diminished cortical T levels.

Reduction in [^{3}H]-T binding following proteolytic treatment of the tissue homogenate indicates that the [^{3}H]-T binding site must be protein in nature. This is a requirement if the

binding site is to act as a receptor and thus have the capacity to induce any second messenger effect triggered by T binding. Although solubilization effects and biochemical characterization using a variety of detergents and protein modifying agents have been published (Brüning and Rommelspacher, 1985a,b, 1989), the exact nature of the high-affinity site labelled by [^{3}H]-T remains unknown.

4.B Effect of chronic administration of antidepressant drugs on [^{3}H]-T binding in rat cortex

Introduction: Having investigated some of the physical and physiological aspects of [^{3}H]-T binding, the next logical step was the investigation of the same binding parameters following the administration of clinically useful psychotropic agents. Since T has been proposed as an etiological factor in a variety of psychiatric disorders, including depression (Dewhurst, 1968a,b; Slingsby and Boulton, 1976), the compounds chosen for study represent two of the classes of compounds used in the treatment of depression, namely the MAO inhibitors and the tricyclic antidepressants. The MAO inhibitors used could be further subclassified as nonselective and selective. Examples of the former are phenelzine and tranylcypromine, two of the most widely prescribed MAO-inhibiting antidepressants (Baker *et al.*, 1988b). Clorgyline and (-)-deprenyl represent selective inhibitors of MAO-A and MAO-B, respectively (Knoll *et al.*, 1965; Johnston, 1968; Neff and Yang,1974). Reports to date state that [^{3}H]-T binding is reduced following chronic administration of the MAO inhibitors clorgyline (Martin *et al.*, 1987) and pargyline (Kellar and Cascio, 1986) and molindone (van Nguyen and Juorio, 1989b). (-)-Deprenyl was found not to exert any effect on [^{3}H]-T binding parameters (Martin *et al.*, 1987).

The tricyclic antidepressants used for this study were the prototypical tricyclic antidepressant imipramine, its desmethyl metabolite desipramine and its chloro-analogue clomipramine. In view of the structural similarity between T and 5-HT (Figure 5) and the 5-HT uptake inhibiting capacity of imipramine (Langer *et al.*, 1983), this tricyclic antidepressant seemed

an obvious choice for the investigation of the effects of tricyclic antidepressant drugs on [^{3}H]-T binding. Another reason for investigating the effects of imipramine on [^{3}H]-T binding was the observation that T may competitively inhibit [^{3}H]-imipramine binding in human platelets (Langer *et al.*, 1983; Segonzac *et al.*, 1985). Desipramine, a potent NA uptake inhibitor, was investigated as it is the major metabolite of imipramine. Clomipramine, the desmethyl metabolite of which is less potent at inhibiting the uptake of NA back into the nerve terminal than is desipramine, is also a more potent 5-HT uptake inhibitor than imipramine (Randrup and Braestrup, 1977; Benfield *et al.*, 1980; Hall and Ogren, 1981). Clomipramine is also becoming the drug of choice for the treatment of obsessive-compulsive disorder (Montgomery, 1980; Flament *et al.*, 1987; Zohar and Insel, 1987).

4.B.1 Non-selective MAO inhibitors: Phenelzine and tranylcypromine

The non-selective MAO inhibitors phenelzine (5 and 10 mg kg^{-1} per d) and tranylcypromine (0.5 and 1 mg kg^{-1} per d) were administered (28 d) to rats via osmotic pumps as described in section 3.A.14. Inhibition of MAO in brain and liver (Table 9) tissue was significant in all treatment groups. Scatchard analyses revealed significant decreases in the [^{3}H]-T B_{max} for phenelzine-treated [$F(2,12)=53.23$] and tranylcypromine-treated [$F(2,9)=13.65$] rat cortical tissue (Figure 9). Single point binding revealed a decrease in [^{3}H]-T binding following tranylcypromine (0.5 mg kg^{-1} per d) treatment [$F(1,14)=5.61$]. [^{3}H]-DHA binding was also significantly decreased following phenelzine [$F(2,34)=12.04$] and tranylcypromine (1 mg kg^{-1} per d) [$F(1,27)=11.21$] (Figure 9). The K_Ds for [^{3}H]-T and [^{3}H]-DHA binding remained unchanged following treatment.

4.B.2 Selective MAO inhibitors: Clorgyline and (-)-deprenyl

The selective MAO inhibitors clorgyline (1 mg kg^{-1} per d) and (-)-deprenyl (1 mg kg^{-1} per d) were administered (14 d) to rats using osmotic minipumps. Scatchard analyses revealed

BRAIN	MAO Inhibition (%)	
Treatment (mg kg^{-1})	MAO-A	MAO-B
Tranylcypromine 0.5	84 (1)*	90 (1)*
Tranylcypromine 1.0	76 (2)*	85 (1)*
Phenelzine 5.0	83 (2)*	89 (1)*
Phenelzine 10.0	85 (2)*	89 (2)*

LIVER	MAO Inhibition (%)	
Treatment (mg kg^{-1})	MAO-A	MAO-B
Tranylcypromine 0.5	92 (1)*	88 (1)*
Tranylcypromine 1.0	87 (1)*	92 (0)*
Phenelzine 5.0	92 (1)*	66 (6)*
Phenelzine 10.0	96 (0)*	86 (6)*

Table 9: Level of MAO inhibition achieved in whole brain (less cortex) [top] and liver [bottom] tissue following a chronic (28 d) regimen of the non-selective MAO inhibitors tranylcypromine and phenelzine. Values represent inhibition relative to vehicle-treated controls. Mean ± (SEM); *: significantly different from control levels.

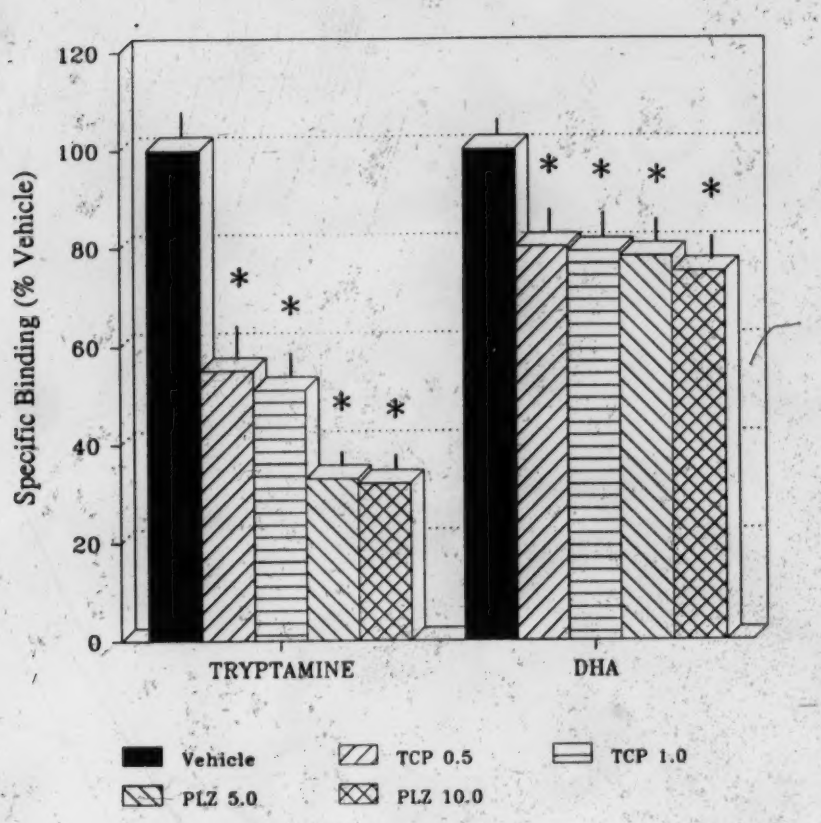


Figure 9: Effect of chronic (28 d) administration of the non-selective MAO inhibitors tranylcypromine (TCP; 0.5 and 1 mg kg^{-1} per d) and phenelzine (PLZ; 5 and 10 mg kg^{-1} per d) on [^{3}H]-T and [^{3}H]-DHA binding to rat cortex. *: significantly different from vehicle control levels ([^{3}H]-T: 310 ± 16 fmol mg^{-1} protein; [^{3}H]-DHA: 89 ± 5 fmol mg^{-1} protein).

decreases in the B_{max} for [^{3}H]-T binding following both MAO inhibitor treatments [$F(2,12)=26.51$ and $F(2,12)=6.24$, for clorgyline and (-)-deprenyl, respectively; Figure 10]. The K_Ds were unchanged following treatment. Preferential inhibition of the MAO isozymes was observed in both brain and liver tissue (Table 10).

4.B.3 Tricyclic antidepressants: Imipramine, desipramine and clomipramine

The tricyclic antidepressants imipramine (30 mg kg^{-1} per d), desipramine (10 mg kg^{-1} per d) and clomipramine (10 mg kg^{-1} per d) were administered to rats (28 d) using osmotic minipumps. Scatchard analyses revealed no change in the B_{max} for [^{3}H]-T binding following imipramine and desipramine treatment [$F(2,13)=0.70$] (Figure 11). The effect of clomipramine on [^{3}H]-T binding was investigated by single-point binding. As with the other tricyclic antidepressants, there was no change in the amount of [^{3}H]-T binding [$F(1,14)=7.12\times10^{-5}$]. The [^{3}H]-DHA B_{max} was decreased following imipramine and desipramine treatment [$F(2,34)=29.36$] (Figure 11). Single-point binding was again used for the investigation of clomipramine effects on [^{3}H]-DHA binding, revealing a significant decrease [$F(1,14)=9.33$]. The K_Ds were unaffected by tricyclic antidepressant treatment. Inhibition of MAO following tricyclic antidepressant treatment was negligible in both brain and liver tissue (Table 11), although the level of MAO inhibition in the liver was slightly raised following clomipramine treatment. Clomipramine has been observed to have a slight but more pronounced effect on MAO-A than does imipramine or amitriptyline (D.L. Murphy unpublished data, 1986, cited in Flament *et al.*, 1987).

4.B.4 [^{3}H]-T and [^{3}H]-IMI binding in human platelets

To confirm the finding that the [^{3}H]-T and [^{3}H]-imipramine binding sites are not the same site (Kaulen *et al.*, 1986), comparative binding was conducted on human platelets, a tissue known to be rich in [^{3}H]-imipramine binding sites (Langer *et al.*, 1980).

Using the single-point binding procedures described in sections 3.D.1.3 and 3.D.3.1, [^{3}H]-T

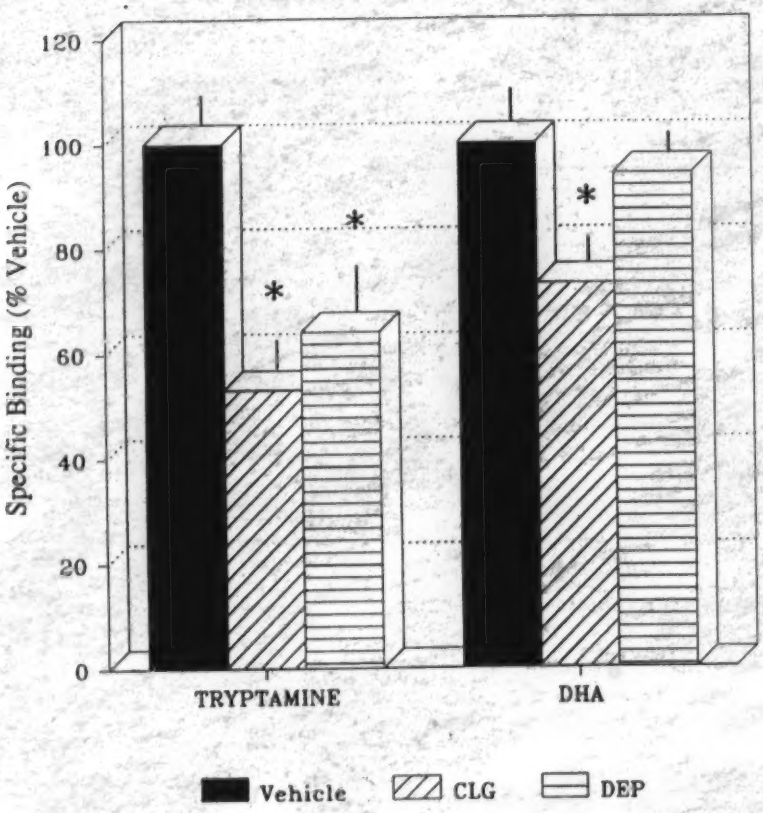


Figure 10: Effect of chronic (14 d) administration of the selective MAO inhibitors clorgyline (CLG; 1 mg kg^{-1} per d) and (-)-deprenyl (DEP; 1 mg kg^{-1} per d) on [^{3}H]-T and [^{3}H]-DHA binding to rat cortex. *: significantly different from vehicle control levels ([^{3}H]-T: 327 ± 14 fmol mg^{-1} protein; [^{3}H]-DHA: 100 ± 9 %dpm mg^{-1} protein).

BRAIN	MAO Inhibition (%)	
Treatment	MAO-A	MAO-B
Clorgyline	85 (2)*	51 (3)*
(-)-Deprenyl	52 (4)*	76 (2)*

LIVER	MAO Inhibition (%)	
Treatment	MAO-A	MAO-B
Clorgyline	57 (5)*	17 (4)*
(-)-Deprenyl	16 (4)*	75 (3)*

Table 10: Level of MAO inhibition achieved in liver tissue following a chronic (14 d) regimen of the selective MAO inhibitors clorgyline (1 mg kg^{-1} per d) and (-)-deprenyl (1 mg kg^{-1} per d). Values represent % inhibition relative to vehicle-treated controls. Mean ± (SEM); *: significantly different from control levels.

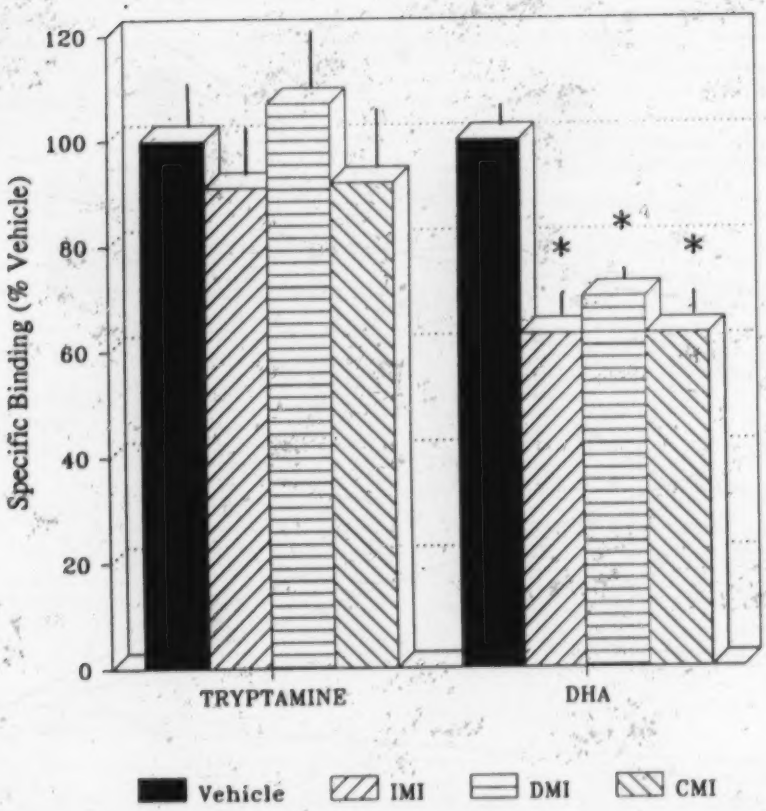


Figure 11: Effect of chronic administration (28 d) of the tricyclic antidepressants imipramine (IMI; 30 mg kg^{-1} per d), desipramine (DMI; 10 mg kg^{-1} per d) and clomipramine (CMI; 10 mg kg^{-1} per d) on [^{3}H]-T and [^{3}H]-DHA binding to rat cortex. *: significantly different from vehicle control levels ([^{3}H]-T: 204 ± 20 fmol mg^{-1} protein; [^{3}H]-DHA: 97 ± 5 fmol mg^{-1} protein).

BRAIN	MAO Inhibition (%)	
Treatment	MAO-A	MAO-B
Imipramine	7 (3)	11 (4)
Desipramine	0 (0)	0 (0)
Clomipramine	2 (1)*	9 (2)

LIVER	MAO Inhibition (%)	
Treatment	MAO-A	MAO-B
Imipramine	5 (4)	12 (8)
Desipramine	0 (2)	0 (3)
Clomipramine	17 (4)*	18 (5)*

Table 11: Level of MAO inhibition achieved in whole brain (less cortex) [top] and liver [bottom] tissue following a chronic (28 d) regimen of the tricyclic antidepressants imipramine (30 mg kg^{-1} per d), desipramine (10 mg kg^{-1} per d) and clomipramine (10 mg kg^{-1} per d). Values represent % inhibition relative to vehicle-treated controls. Mean ± (SEM); *: significantly different from control levels.

and [^{3}H]-imipramine binding parameters, respectively, were investigated in human platelet preparations. It was shown that although [^{3}H]-imipramine binding was present in this tissue (approximately 222.3 fmol/mg protein), [^{3}H]-T binding was not.

Discussion: The drugs investigated in this study induced a decrease in the B_{max} for [^{3}H]-DHA binding which is in accord with published findings (Vetulani *et al.*, 1976; Peroutka and Snyder, 1980; Maj *et al.*, 1984; Murphy *et al.*, 1984; Snyder and Peroutka, 1984). The MAO-inhibiting drugs, whether non-selective or selective, induced a decrease in the amount of [^{3}H]-T binding. As mentioned previously, a similar effect of clorgyline on [^{3}H]-T binding has been reported elsewhere (Martin *et al.*, 1987). The decreases in [^{3}H]-T binding following the other MAO inhibitor treatments are all novel findings. MAO has been shown to have a high affinity for T (Philips and Boulton, 1979; Sullivan *et al.*, 1986) and thus inhibition of this enzyme would be expected to affect [^{3}H]-T binding by altering the availability of endogenous T. The observation that (-)-deprenyl induced a decrease in [^{3}H]-T binding is in contradiction to the findings of the study done by Martin *et al.* (1987). In the latter study (-)-deprenyl was administered *ip* whereas in the present study (-)-deprenyl was administered *sc*. Waldmeier *et al.* (1981) reported finding a greater potency of the selective MAO inhibitors clorgyline and (-)-deprenyl in brain when administered *sc*. These authors have proposed that this phenomenon might be due to a greater first-pass metabolism of the drugs following oral or *ip* administration relative to *sc* administration. Although clorgyline and (-)-deprenyl are selective for MAO isozymes, the level of inhibition of either MAO isozyme in the present study was statistically different from control levels following either treatment (Table 10).

Administration of tricyclic antidepressant drugs did not affect [^{3}H]-T binding. Kaulen and colleagues (1986) had previously shown that the [^{3}H]-T binding site was distinct from the [^{3}H]-imipramine site. This is of interest since Langer and colleagues (Langer *et al.*, 1983; Segonzac *et al.*, 1985) found that T displaced imipramine at the [^{3}H]-imipramine binding

site. The small active component involved in T uptake into brain tissue and platelets is also known to be affected by 5-HT uptake inhibitors, including tricyclic antidepressants (Dyck, 1984a,b; Segonzac *et al.*, 1984). If T were to exert some of its actions at the [^{3}H]-imipramine-labelled 5-HT uptake site (Langer and Briley, 1981), then chronic administration of imipramine would theoretically, through competition for the binding site, reduce [^{3}H]-T binding. This was, however, not the case. The difference in findings between the present investigation and the studies by Langer *et al.* (1983) and Segonzac *et al.* (1985) could be attributed to the large quantity of T required to displace [^{3}H]-imipramine at the binding site (i.e. 3300-3500 nM) in these last two studies.

Drug treatments in this section were validated by comparing the levels of MAO inhibition and the extent of [^{3}H]-DHA binding. MAO-inhibiting drugs resulted in a significant change in MAO activity (type-A or -B or both, depending on the selectivity of the MAO inhibitor) as well as in a significant reduction in the amount of [^{3}H]-DHA binding. On the other hand, tricyclic antidepressant treatment resulted in a significant reduction in the B_{max} for [^{3}H]-DHA binding without significantly affecting MAO activity.

4.C Functional assessment of T and presynaptic D_2 DA receptors in rats having received chronic antidepressant treatment

4.C.1 T receptors

Introduction: This study was conducted to evaluate the relative effects of both tricyclic and MAO-inhibiting antidepressant drugs on the functional responsiveness of [^{3}H]-T receptors. The functional approach applied during this study was based on previous work demonstrating behavioural responsiveness to adrenergic receptor agonists such as clonidine (α_2) and salbutamol (β_2) following chronic antidepressant treatment (Spyraki and Fibiger, 1980; Cohen *et al.*, 1982; Przegalinski *et al.*, 1984; Greenshaw *et al.*, 1988; McManus *et al.*, 1990). The effect of 21 days of administration of imipramine, desipramine, clomipramine, tranylcypromine, and clorgyline on a functional measure of [^{3}H]-T receptor sensitivity,

i.e. effects of T on locomotor activity in rats in comparison with the number and affinity of [^{3}H]-T binding sites (see section 4.B), were assessed. It was hypothesized that the locomotor effects of exogenous T would be attenuated in rats having received a drug treatment which induced a decrease in the [^{3}H]-T B_{max}.

Results:

4.C.1.1 Pilot study

Treatment with an MAO inhibitor blocks the catabolism of T, which is rapid *in vivo* (Durden and Philips, 1980; Juorio and Durden, 1984; Sullivan *et al.*, 1986; Baker *et al.*, 1988b). Any experiment comparing MAO inhibitor- and tricyclic antidepressant-induced effects on T-mediated function would thus have a confounding factor in the experimental design. As this was the case for the following behavioural experiment, it was deemed necessary to pretreat the test animals, immediately prior to testing, with an MAO inhibitor able to alter MAO activity to the same extent in all treatment groups. The MAO inhibitor chosen for this purpose was pargyline. Preliminary tests were conducted to determine the appropriate dose for pargyline treatment. [^{3}H]-T binding was also investigated during these preliminary tests to confirm the lack of acute pargyline-induced effects on [^{3}H]-T binding (Kellar and Cascio, 1986).

The effect of acute doses of pargyline (50 and 100 mg kg^{-1}, *ip*) was investigated with regard to MAO activity and [^{3}H]-T binding. Both isozymes of MAO were significantly inhibited ($\geq$82 % inhibition) following either dose of pargyline [$F(2,16)=134.70$ and $F(2,16)=123.56$, for MAO-A and -B, respectively] (Table 12). [^{3}H]-T binding, determined by single point binding, remained unchanged [$F(2,16)=0.09$] from control levels following administration of either dose of pargyline (Table 13). As the 50 mg kg^{-1} dose of pargyline was effective at significantly inhibiting MAO, the concurrent administration of pargyline with T (0.03, 0.1 and 0.3 mmol kg^{-1}, *sc*) was subsequently investigated for effects on MAO activity.

Neither of the T doses significantly changed levels of MAO inhibition observed after par-

	MAO Inhibition (%)	
Treatment	MAO-A	MAO-B
Pargyline 50	88 (1)*	82 (1)*
Pargyline 100	90 (1)*	86 (1)*

Table 12: Level of MAO inhibition achieved in whole brain (less cortex) tissue following acute pargyline (50 and 100 mg kg^{-1} *ip*, 24 h) treatment. Values represent % inhibition relative to vehicle-treated controls. Mean ± (SEM); *: significantly different from control levels.

Treatment	[^{3}H]-T Binding
Vehicle	4247 (104)
Pargyline 50	4118 (281)
Pargyline 100	4161 (201)

Table 13: Amount of [^{3}H]-T binding, dpm per mg protein, in cortex following acute pargyline (50 and 100 mg kg^{-1} *ip*, 24 h) treatment. Mean ± (SEM).

gyline alone [F(3,36)=0.37 and F(3,36)=0.33, for MAO-A and -B, respectively] (Table 14). Rats were pretreated with pargyline (50 mg kg^{-1} *ip*, 24 h), treated with T (0.03, 0.1 or 0.3 mmol kg^{-1} *sc*, 0 min) and put into activity cages. A dose-dependent decrease in locomotor activity was observed in the three activity categories measured, i.e. total [F(3,36)=16.13], consecutive [F(3,36)=11.75] and vertical [F(3,36)=36.53] activity (Table 15).

The results in section 4.C.1 indicated that a 50 mg kg^{-1} (*ip*, 24 h) pretreatment dose of pargyline followed by T (0.1 mmol kg^{-1} *sc*, 0 min) would be sufficient for inducing both a significant inhibition of MAO activity and a locomotor suppressant effect, without affecting [^{3}H]-T binding.

4.C.1.2 Functional assessment of administered T

Tranylcypromine (0.5 and 1.0 mg kg^{-1} per d), clorgyline (1.0 mg kg^{-1} per d), imipramine (30 mg kg^{-1} per d), and clomipramine and desipramine (10 mg kg^{-1} per d, each) were administered (28 d) to rats using osmotic minipumps. [^{3}H]-T binding had previously been shown to be decreased following chronic treatment with MAO inhibitors (see sections 4.B.1-2) but not with tricyclic antidepressant drugs (see section 4.B.3).

In this experimental design, the test rats acted as their own controls. On day 20 of the drug treatment the rats were pretreated with pargyline. On day 21, locomotor activity was measured (for a maximum of 15 min so as to avoid habituation). The rats were removed from the test cages, replaced into their respective housing cages, and later (after 4 h) reintroduced to the same test cages, following T treatment (0 min). This experimental design allowed for baseline (pretreatment) locomotor activity measurements, as well as treatment locomotor activity measurements in the same rats. The results of the probe were expressed as percentages (i.e. treatment locomotor activity as a percentage of the respective baseline locomotor activity).

The effects of this probe on total locomotor activity are depicted in Figure 12. A significanty locomotor effect of drug [F(6,56)=10.79] and time [F(2,112)=13.83] was observed. Sub-

	MAO Inhibition (%)	
Treatment	MAO-A	MAO-B
Pargyline + 0.0 T	88 (1)*	90 (1)*
Pargyline + 0.03 T	88 (1)*	90 (1)*
Pargyline + 0.1 T	89 (1)*	91 (1)*
Pargyline + 0.3 T	88 (1)*	91 (1)*

Table 14: Level of MAO inhibition achieved in whole brain tissue following acute administration of pargyline (50 mg kg^{-1} *ip*, 24 h) and doses of T (in mmol kg^{-1} *sc*, 15 min). Values represent % inhibition relative to vehicle-treated controls. Mean ± (SEM); *: significantly different from control levels.

	Activity		
Treatment	Total	Consecutive	Vertical
Pargyline + 0.0 T	4407 (198)	483 (24)	294 (19)
Pargyline + 0.03 T	3411 (273)*	393 (16)*	134 (26)*
Pargyline + 0.1 T	2722 (232)*	349 (23)*	66 (13)*
Pargyline + 0.3 T	2408 (164)*	325 (17)*	59 (10)*

Table 15: Activity measured during a half-hour period following the administration of pargyline (50 mg kg^{-1} *sc*, 24 h) in combination with doses of T (in mmol kg^{-1} *sc*, 0 min). Mean ± (SEM); *: significantly different from control levels.

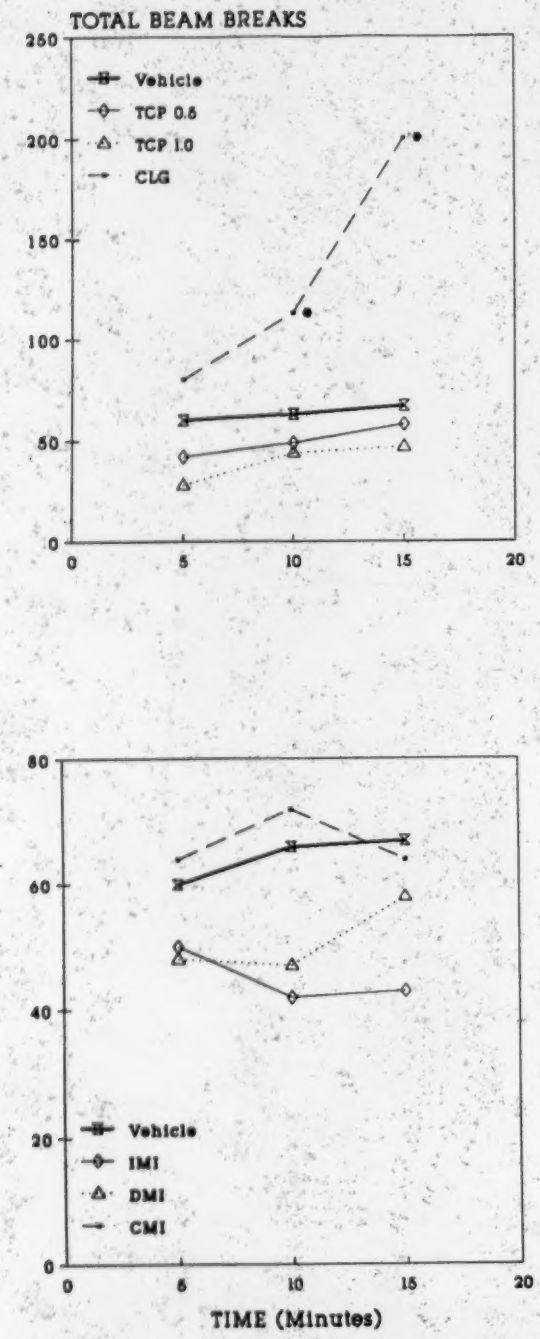


Figure 12: Effect of chronic administration of the antidepressant drugs TCP, CLG (top), IMI, DMI and CMI (bottom) on T-induced locomotor activity in the rat (expressed as % vehicle day). See text for details; *: significantly different from vehicle controls.

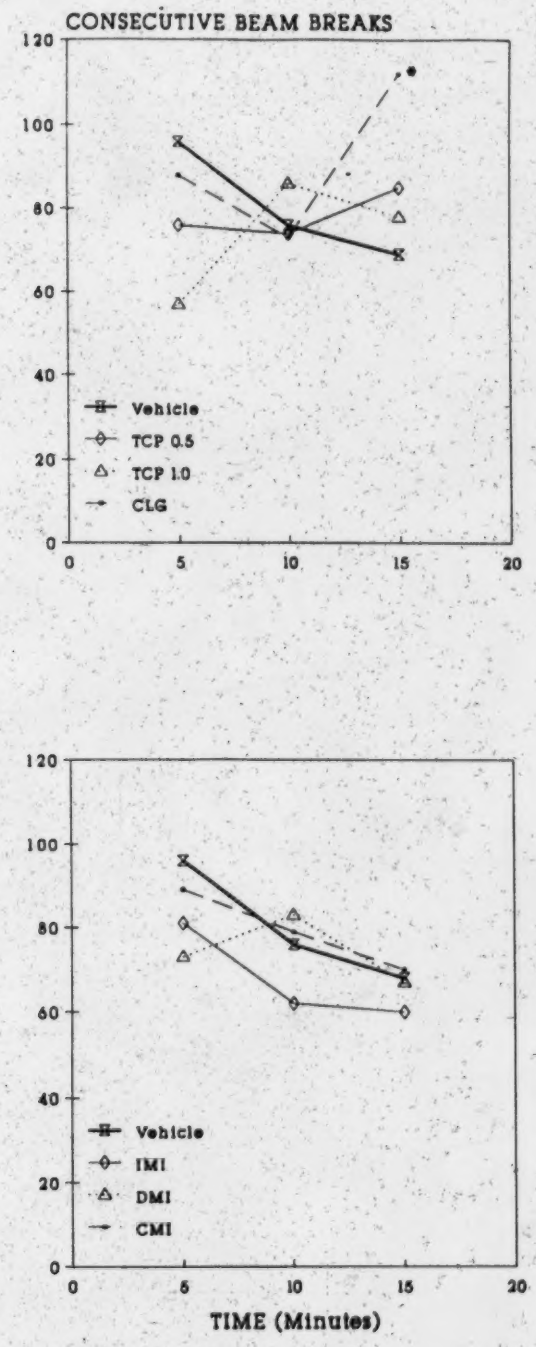


Figure 13: Effect of chronic administration of the antidepressant drugs TCP, CLG (top), IMI, DMI and CMI (bottom) on T-induced locomotor activity in the rat (expressed as % vehicle day). See text for details; *: significantly different from vehicle controls.

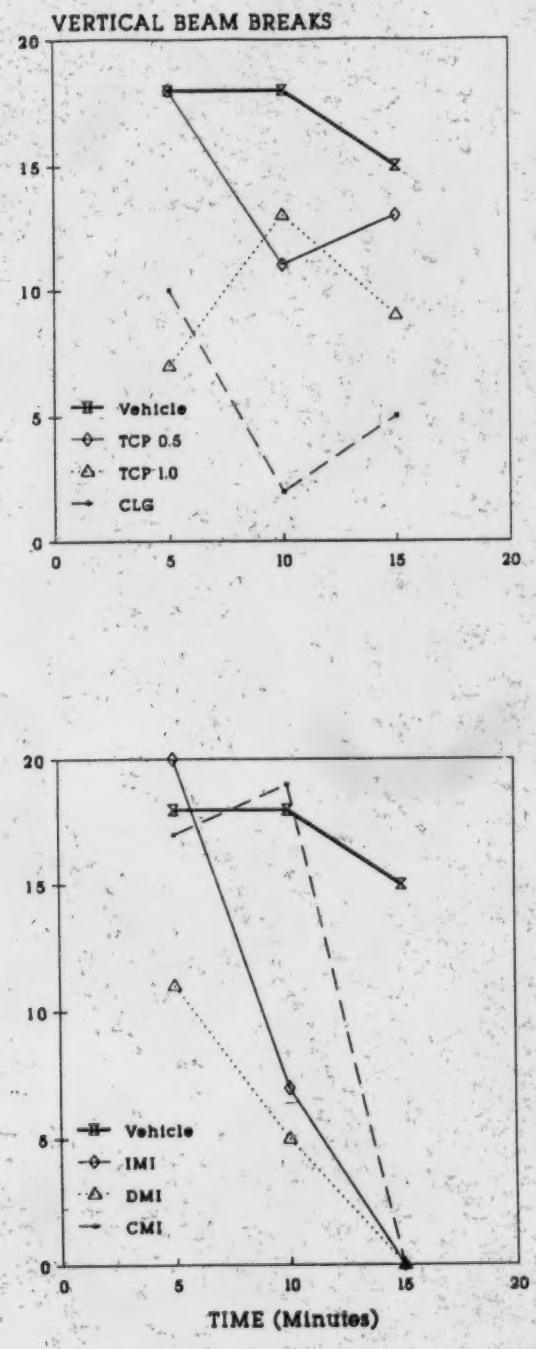


Figure 14: Effect of chronic administration of the antidepressant drugs TCP, CLG (top), IMI, DMI and CMI (bottom) on T-induced locomotor activity in the rat (expressed as % vehicle day). See text for details; *: significantly different from vehicle controls.

	MAO Inhibition (%)	
Treatment	MAO-A	MAO-B
Pargyline	87 (0)*	92 (0)*
Pargyline + CLG	87 (1)*	92 (0)*
Pargyline + TCP[1]	89 (6)*	95 (0)*
Pargyline + TCP[2]	86 (1)*	89 (1)*
Pargyline + IMI	89 (1)*	92 (0)*
Pargyline + DMI	87 (1)*	92 (1)*
Pargyline + CMI	89 (1)*	93 (0)*

Table 16: Level of MAO inhibition achieved in whole brain tissue following acute administration of pargyline (50 mg kg^{-1} *ip*, 24 h) and antidepressant drug treatment. Values represent % inhibition relative to vehicle-treated controls. Mean ± (SEM); *: significantly different from control levels. CLG: clorgyline (1mg kg^{-1}); TCP[1]: tranylcypromine (0.5 mg kg^{-1}); TCP[2]: tranylcypromine (1.0 mg kg^{-1}): IMI: imipramine (30 mg kg^{-1}); DMI: desipramine (10 mg kg^{-1}); CMI: clomipramine (10 mg kg^{-1}).

sequent analysis demonstrated this to be due to an effect of clorgyline at 10 and 15 min. An interaction between drug and time was also evident [F(12,112)=7.57]. With regard to consecutive activity, only clorgyline treatment had significant effects (Figure 13). An increase in locomotor activity was observed in this activity category as a significant interaction term (clorgyline x time, at 15 min) in the ANOVA [F(12,112)=2.76]. Vertical activity was decreased in a time-dependent manner [F(2,112)=5.53] (Figure 14). Activities of MAO-A and -B were inhibited (in all pargyline-treated animals) to the same extent as observed in the pilot study (Table 16).

Discussion: The hypothesized attenuation of T-induced locomotor suppression in MAO inhibitor treated animals was not observed in this last experiment. The conclusion attained from these results is that exogenous T does not exert its behavioural effects simply *via* an action on the [^{3}H]-T binding site. This was demonstrated by the differential response to exogenous T following clorgyline and tranylcypromine treatment, both of which were shown to alter [^{3}H]-T binding site density (see sections 4.B.1-2). The locomotor stimulant effect following exogenous T application observed in rats chronically treated with clorgyline implies an action of this MAO inhibitor which lies outside of the spectrum of action of the other MAO inhibitors studied here.

4.C.2 Combined T and amphetamine administration: Behavioural effects

Introduction: The stimulant effect observed during the T probe with the clorgyline treated rats was quite unexpected and interesting. Clorgyline, being a selective MAO-A inhibitor, increases the levels of many amines besides T, including NA, 5-HT and DA. DA, a compound known to be a mixed substrate for MAO in the rat (Stenstrom *et al.*, 1985), is also known to be involved in the stimulant effects of a variety of compounds, including amphetamine and apomorphine (Fray *et al.* 1980). It was therefore hypothesized that the stimulant effect observed in the clorgyline-treated animals might well be due to an interaction

between exogenous T and a possible increase in the synaptic availability of endogenous DA.

Results: To test the hypothesis T (0.1 mmol kg^{-1} *sc*, 0 min) and amphetamine (0.75 mg kg^{-1} *sc*, 10 min) were administered either alone or in combination. Amphetamine was used because of its ability to release DA from the nerve terminal (Kuczenski, 1983; Zetterstrom *et al.*, 1983; Kuczenski *et al.*, 1990), as well as for its motor stimulant effect (Fray *et al.*, 1980). Pargyline (50 mg kg^{-1} *sc*, 24 h) was used as a pretreatment in all test groups. Total locomotor activity was measured for 60 min using the activity test cages.

Amphetamine induced a significant stimulant effect [$F(1,44)=10.10$], whereas T induced a decrease in locomotor activity [$F(1,44)=9.70$], consistent with previous findings (Figure 15). There was no interaction between these two compounds when given concurrently to rats [$F(1,44)=0.33$], although there was a time x T x amphetamine interactive effect [$F(5,220)=2.42$].

Discussion: The findings of this last study indicate that the stimulatory effects observed following T administration to rats chronically treated with clorgyline do not appear to be a result of an action of T on released DA. Further investigation on these possible modulatory aspects of T are needed.

4.C.3 Presynaptic D_2 DA receptors

Introduction: This experiment was undertaken to evaluate the effects of apomorphine on locomotor activity in rats having received a chronic drug treatment and is based on the approach discussed earlier (see section 4.C.1 on the functional assessment of T receptors). Low doses of apomorphine exert their behavioural effects by inhibiting DA synthesis through its interactions with DA (presynaptic D_2) autoreceptors (Carlsson, 1975; Aghajanian and Bunney, 1977; Mogilnicka and Klimek, 1977; Roth, 1984). It was also of interest to compare the effects of a low dose of apomorphine following a drug treatment similar to that used during the T probe so as to investigate the possibility that both T and low-dose apomorphine

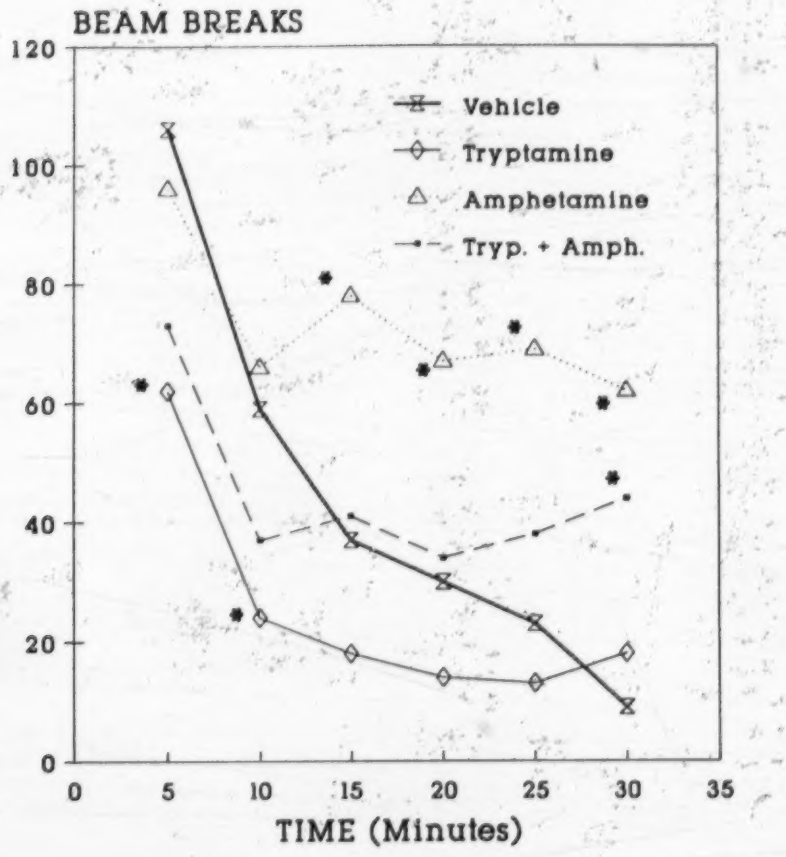


Figure 15: Effect of administration of T and/or amphetamine on the total spontaneous locomotor activity in the rat. See text for details; *: significantly different from vehicle controls.

exert their behavioural effects *via* an action at the presynaptic D_2 DA receptors. It had previously been shown that *iv* T inhibited the firing rate of caudate neurons in a manner similar to that observed following *iv* apomorphine; however, the effects of apomorphine were reversed by haloperidol, whereas those of T were not (van Nguyen *et al.*, 1989). Thus, T and apomorphine do not act at the same receptor postsynaptically. T has been shown to act on the release and uptake of DA from the nerve terminal (Baker and Yasensky, 1981; Ennis *et al.*, 1981; Marien *et al.*, 1987) and thus any concordance between the effects observed in these two probes might help to elucidate the function of T at a molecular (receptor) level. It was hypothesized that, if T and DA exerted some of their effects *via* closely associated presynaptic receptors, the sedative (and thus locomotor suppressant) effects of low-dose apomorphine would remain unaffected by tranylcypromine, imipramine, clomipramine and desipramine treatment but would be altered in clorgyline treated rats, as observed during the T probe.

Tranylcypromine, clorgyline, imipramine, clomipramine and desipramine (doses as described in section 4.C.1.2) were administered (28 d) to rats using osmotic minipumps.

Results:

4.C.3.1 Pilot study

Apomorphine (50 $\mu g\ kg^{-1}$ in 0.2 % ascorbate *sc*, 5 min) was administered to rats and locomotor activity was measured using the activity test cages. This dose of apomorphine was shown to be sedative with regard to total [$F(1,18)=25.20$], consecutive [$F(1,18)=18.98$] and vertical [$F(1,18)=19.93$] locomotor activity (Table 17).

4.C.3.2 Functional assessment of administered apomorphine

The experimental design for this probe was very similar to the design for the T probe (see section 4.C.2.1), as rats acted as their own controls. Pargyline pretreatment was not necessary, however, and thus was excluded. Between days 20 and 21 of drug administration the effects of apomorphine on locomotor activity were assessed. Half of each drug treatment

	Activity		
Treatment	Total	Consecutive	Vertical
Vehicle	1006 (318)	126 (40)	87 (28)
Apomorphine	332 (105)*	52 (23)*	39 (12)*

Table 17: Activity measured during a half-hour period following the administration of apomorphine (50 $\mu g\ kg^{-1}$ *sc*, 5 min) in a 2 mg ml^{-1} solution of ascorbic acid. Mean ± (SEM); *: significantly different from vehicle levels.

group of animals received apomorphine on day 20 and the distilled water vehicle on day 21. This order of drug/vehicle testing was reversed for the remaining animals to control for possible order effects. Locomotor activity was measured for 30 min. This counterbalanced experimental design allowed for baseline measurements in the same rats. The results of the probe were expressed as percentages (i.e. treatment locomotor activity as a percentage of the respective baseline locomotor activity).

The effects of this probe on total activity are depicted in Figure 16. A time-dependent effect was evident [F(2,84)=82.03]. As an oversight in the original experimental design resulted in the omission of a desipramine treatment group, a separate experiment studying the effect of this drug was conducted. Desipramine pretreatment attenuated the locomotor suppressant effect of apomorphine [F(1,18)=5.95]. A significant interaction term (drug x time, at 5 min) was also observed [F(2,36)=6.83]. Consecutive activity was altered in a time-dependent manner [F(2,84)=43.34]. The desipramine experiment revealed a time-dependent effect [F(2,36)=38.55] which again gave a significant interaction (drug x time, at 5 min) term [F(2,36)=3.37] (Figure 17). A time-dependent effect on vertical activity was observed [F(2,84=31.06]. The desipramine experiment also revealed a time-dependent effect [F(2,36)=33.28] (Figure 18).

Discussion: A number of conclusions follow from the testing in this functional probe. The lack of concordance between the results of the T probe and the apomorphine probe indicates that T does not exert its effects directly through activation of, nor through cooperativy with, the presynaptic D_2 DA receptor. In addition, the attenuation of the response to apomorphine by desipramine pretreatment implies a down-regulation of the DA D_2 presynaptic receptor by this tricyclic antidepressant drug.

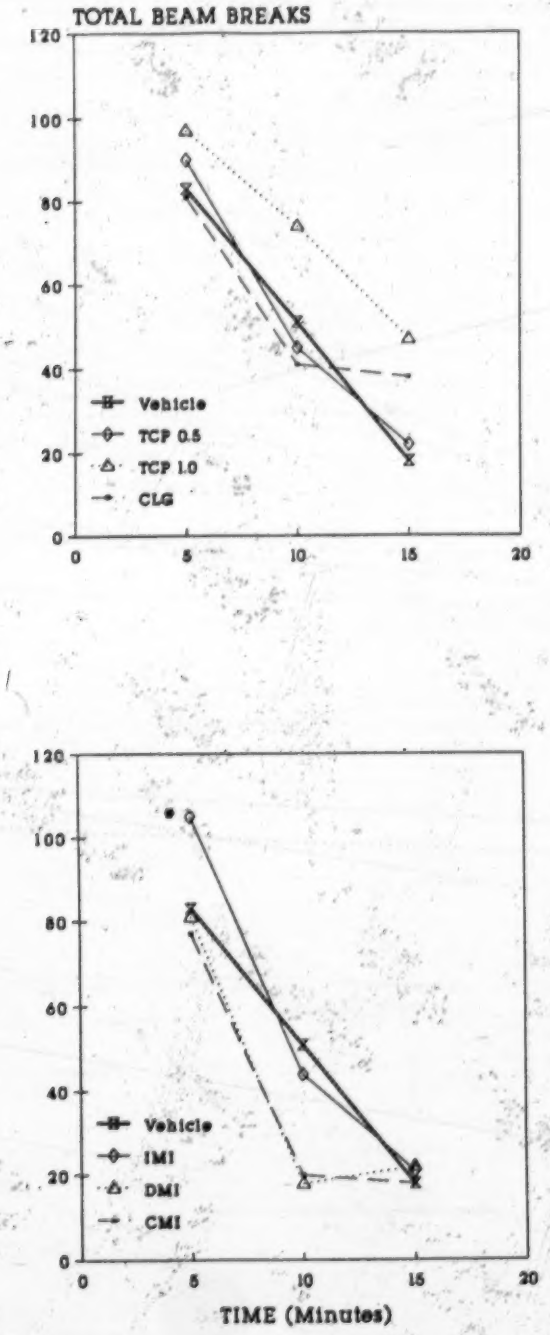


Figure 16: Effect of chronic administration of the antidepressant drugs TCP, CLG (top), IMI, DMI and CMI (bottom) on APO-induced locomotor activity in the rat (expressed as % vehicle day). See text for details; *: significantly different from vehicle controls.

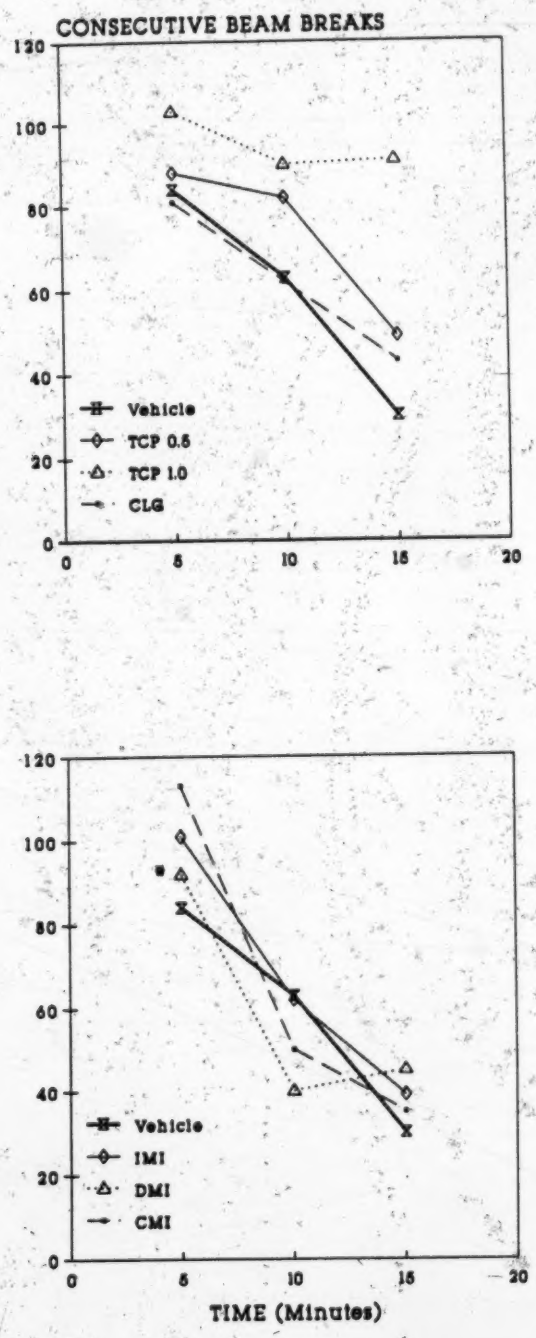


Figure 17: Effect of chronic administration of the antidepressant drugs TCP, CLG (top), IMI, DMI and CMI (bottom) on APO-induced locomotor activity in the rat (expressed as % vehicle day). See text for details; *: significantly different from vehicle controls.

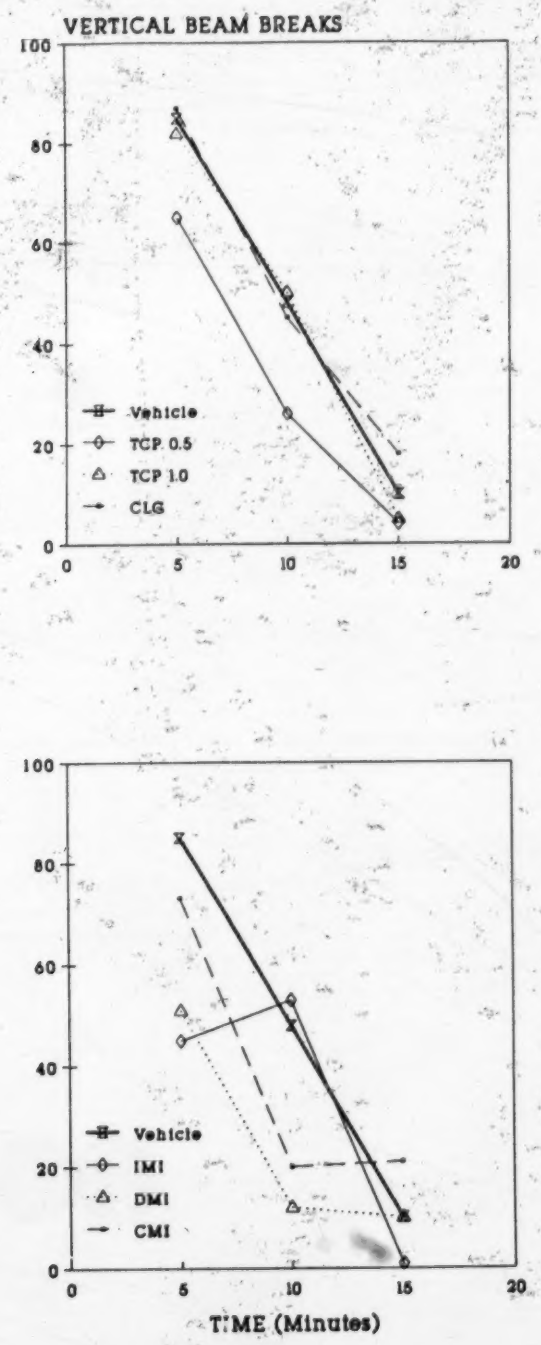


Figure 18: Effect of chronic administration of the antidepressant drugs TCP, CLG (top), IMI, DMI and CMI (bottom) on APO-induced locomotor activity in the rat (expressed as % vehicle day). See text for details; *: significantly different from vehicle controls.

4.D Chronic administration of clorgyline and clomipramine: Brain levels of biogenic amines and selected acid metabolites

Introduction: The effects observed with clorgyline during the T probe (see Section 4.C.1), and the lack of similar effects during the apomorphine probe (see Section 4.C.2), posed interesting questions regarding interactions between T and the metabolism of the classical biogenic amines, DA and 5-HT, following chronic antidepressant drug treatment. For purposes of contrast and comparison the potent 5-HT uptake blocker, clomipramine, was included in the study. Another consideration in this study is the lack of data concerning the effects of chronic administration of antidepressant drugs on regional brain amine metabolism. These effects are of interest because of the observed time lag between the onset of drug therapy and reversal of the depressed mood (Oswald *et al.*, 1972; Quitkin *et al.*, 1986; Post *et al.*, 1987). The two drugs chosen for this investigation represent two important classes of antidepressant drugs, the MAO inhibitors and the tricyclic antidepressants.

Results:

Clorgyline (1 mg kg^{-1} per d) and clomipramine (5 mg kg^{-1} per d) were administered (28 d) to rats using osmotic minipumps. The frontal cortex (FC), hippocampus (HIPPO), caudate nucleus (CN) and hypothalamus (HYPO) were assayed for biogenic amine (NA, DA and 5-HT) and acid metabolite (DOPAC, HVA and 5-HIAA) content using HPLC-ECD (see section 3.A.3).

The effects of clorgyline on the regional metabolism of the biogenic amines listed above are depicted in Figure 19. NA levels were increased in all regions assayed [$F(2,14)=73.45$, $F(2,15)=34.85$, $F(2,15)=69.22$ and $F(2,12)=155.89$) for the FC, HIPPO, CN and HYPO, respectively], as were the levels of 5-HT [$F(2,14)=70.75$, $F(2,15)=296.57$, $F(2,15)=77.90$ and $F(2,12)=403.68$ for the four regions listed previously]. DA levels were significantly increased only in the CN [$F(2,15)=72.47$]. Acid metabolite levels were differentially decreased in the regions assayed. Both the clorgyline and clomipramine treatments reduced

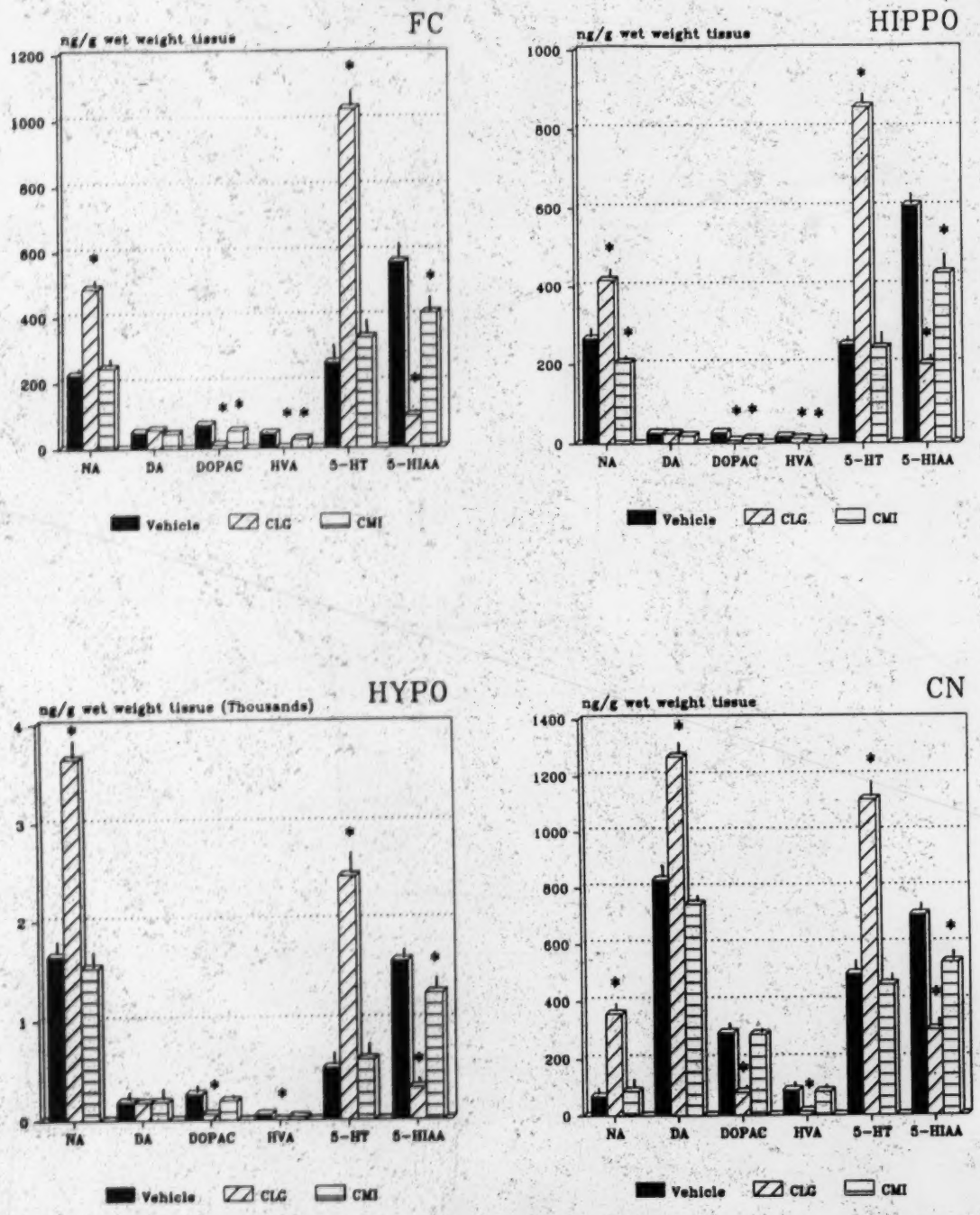


Figure 19: Effect of chronic clorgyline (CLG: 1 mg kg^{-1} per d; 28 d) and clomipramine (CMI: 5 mg kg^{-1} per d; 28 d) administration on levels of NA, DA, DOPAC, HVA, 5-HT and 5-HIAA in the frontal cortex (FC), hippocampus (HIPPO), hypothalamus (HYPO) and caudate nucleus (CN) of the rat. Scale for DA, DOPAC and HVA levels in CN is 0.1. See text for details; (*): significantly different from vehicle control levels.

levels of the DA acid metabolites, DOPAC [F(2,14)=38.46 and F(2,15)=7.58] and HVA [F(2,14)=38.98 and F(2,15)=3.45], in the FC and HIPPO, respectively. In the CN and HYPO respectively, the levels of DOPAC [F(2,15)=43.50 and F(2,12)=20.05] and HVA [F(2,15)=40.20 and F(2,12)=8.03] were reduced following clorgyline treatment only. The levels of 5-HIAA were significantly decreased in all regions assayed [F(2,14)=53.56, F(2,15)=58.91, F(2,15)=79.53 and F(2,12)=58.12, for the FC, HIPPO, CN and HYPO, respectively]. Levels of 5-HT remained unchanged following clomipramine treatment (Figure 19).

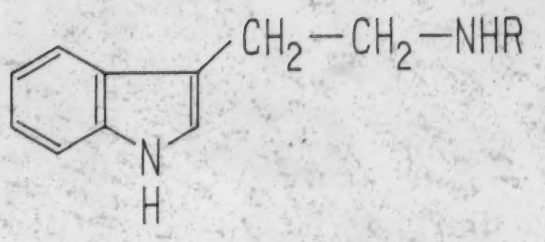
Discussion: The effects of clorgyline and clomipramine on regional brain amine metabolism yielded some interesting results. This study indicated that clorgyline exerts similar effects on amine levels following a 28 d treatment (present study) as it does following a 2 week treatment (O'Regan *et al.*, 1987). The latter study yielded similar levels of the parent compounds and their respective acid metabolites in the striatum and HYPO. The effects of clorgyline are not limited to changes in the metabolism of amines for which MAO-A has a high affinity (ie. T, 5-HT). DA metabolism was also altered and could thus prove to be of importance when considering the possible mode of action of clorgyline. Clorgyline is not known to be extensively metabolized, thereby eliminating a secondary effect of the drug attributable to a metabolite. Clomipramine, however, does undergo metabolism via pathways common to other tertiary tricyclic antidepressants. Clomipramine itself is known to inhibit DA, as well as 5-HT, uptake (Randrup and Braestrup, 1977). In addition, Kurata *et al.* (1986) have demonstrated regional differences in clomipramine concentrations, with the frontal cortex and hippocampus displaying the highest concentrations. This may explain the effect on DA metabolism observed in these two regions following clomipramine administration. Its hydroxyl and desmethyl metabolites have been detected in plasma following oral administration of clomipramine.HCl to humans (Flament *et al.*, 1987) and have been shown to inhibit 5-HT and NA uptake, respectively (Benfield *et al.*, 1980). The effects of clomipramine on amine metabolism may thus be due in part to secondary effects

resulting from its own metabolism.

The finding that DA metabolism was differentially altered following clorgyline and clomipramine treatment might explain the observed differences between the effects induced by these two drugs during the T probe. Although clorgyline did not induce an increase in DA levels in the FC, HYPO and HIPPO, a significant increase in DA content was observed in the CN (Figure 19). Altar and co-workers (1986) proposed that T in the nucleus accumbens, a structure intimately related to the CN, might interact with DA to modulate locomotor activity. This was substantiated by a study conducted by Marien *et al.* (1987) in which injections of α,α-[^{2}H]-T into the nucleus accumbens produced motor stimulation comparable to that observed following similar applications of amphetamine or DA (Pijnenburg *et al.*, 1976; Svensson and Ahlenius, 1983). The observation, in the present study, that clomipramine treatment did not induce a similar increase in caudate nucleus DA levels might therefore explain the different behavioural effects of exogenous T in clorgyline- and clomipramine-treated animals.

4.E Functional assessment of the T system in rats having received acute clorgyline or clomipramine treatment: Locomotor and neurochemical effects

Introduction: As stated previously, T is subject to rapid catabolism by MAO *in vivo* (Durden and Philips, 1980; Juorio and Durden, 1984; Sullivan *et al.*, 1986), making it very difficult to deliver T to its site of action without it undergoing extensive metabolism. The use of a prodrug might alleviate this problem. In this study CET was the prodrug which, *via* the theoretical cleavage of the cyanoethyl moeity, would make T more readily available (Figure 20). The use of an MAO-inhibiting drug as a blocking agent in the catabolism of T is also of importance although selectivity can not be guaranteed, as MAO inhibitors affect levels of many of the biogenic amines (Campbell *et al.*, 1979; Cohen *et al.*, 1985; O'Regan *et al.*, 1987). The fact that T is a substrate for MAO-A and the specificity of clorgyline as a MAO-A



R = H Tryptamine

R = CH_2CH_2CN N-2-Cyanoethyltryptamine

R = $CH_2C{\equiv}CH$ N-Propargyltryptamine

Figure 20: **Structures of T and the N-2-cyanoethyl and N-propargyl analogues of T. The latter two compounds are potential prodrugs (delivery vehicles) for T.**

inhibitor, made clorgyline the drug of choice for the purposes of this investigation. A separate study using clomipramine as a pretreatment was conducted to investigate the action of this compound on T-induced neurochemical and behavioural effects. It was also considered of interest to compare the effects of acute clorgyline and clomipramine treatments on T-mediated effects to those observed following chronic drug treatment (see Section 4.C.1). A separate investigation of the locomotor effects following pretreatment with (-)-deprenyl was also undertaken to determine the specificity of T as a substrate for MAO.

It was hypothesized that the locomotor effects of CET would be similar to those elicited by T and that the effects of concurrent administration of clorgyline (and thus MAO inhibitor-induced potentiation) or clomipramine would result in similar effects in both T- and CET-treated groups, if CET were to exert its effect simply by increasing the availability of T. Brain and liver T levels, as well as MAO inhibition, were assessed in order to elucidate the effectiveness of CET as a prodrug. A preliminary investigation of the neurochemical and behavioural properties of the N-propargyl analogue of T (Figure 20) was also conducted.

Results: In the following sections (i.e. 4.E.1-5) the term 'dose' implies doses of the compound (i.e. T or CET) being investigated in the particular section.

4.E.1 Acute administration of T, either alone or in combination with clorgyline: Locomotor effects

Rats having received a pretreatment injection of clorgyline (1 mg kg^{-1} *sc*, 4 h) followed by an injection of T (0.03, 0.1 or 0.3 mmol kg^{-1} *sc*, 0 min) were placed in locomotor activity cages (see section 3.A.4 and 3.D.10) for a 30 min test period.

Total locomotor activity was decreased in a dose- and time-dependent manner [$F(3,72)=6.79$ and $F(5,360)=172.04$, respectively]. The dose-dependent effect, although not evident following vehicle pretreatment (Figure 21, top), was potentiated by clorgyline pretreatment [clorgyline x doses, $F(3,72)=4.13$] (Figure 21, bottom). Consecutive locomotor activity was also decreased in a dose- [$F(3,72)=5.34$] and time-dependent [$F(5,360)=62.06$] manner

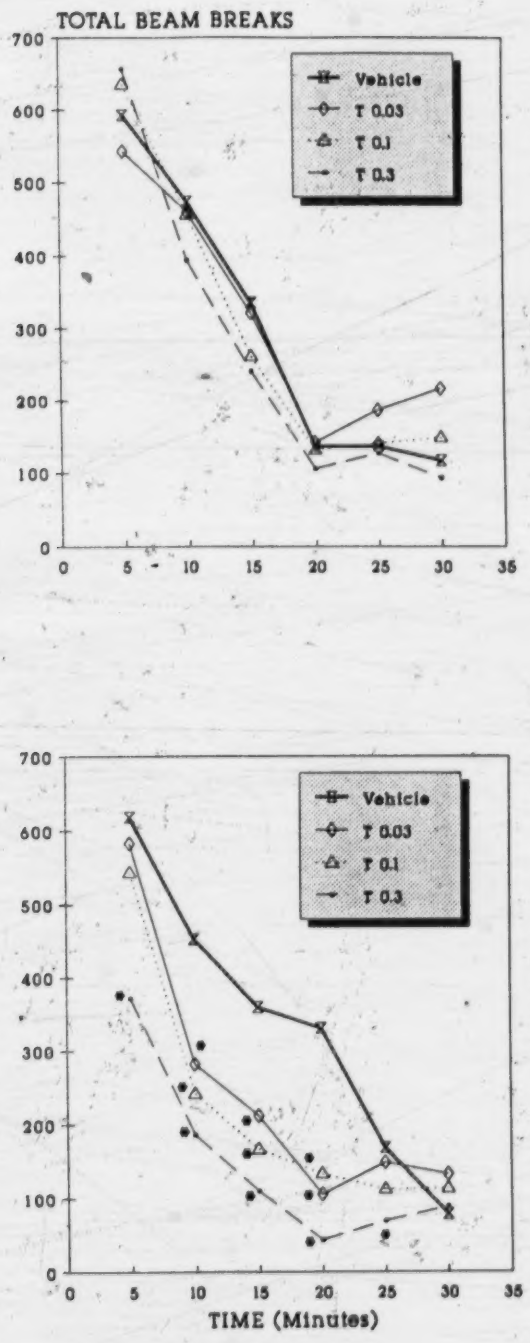


Figure 21: Effect of T administration (in mmol kg^{-1} *sc*, 0 min) either alone (top) or in combination (bottom) with clorgyline (1 mg kg^{-1} *sc*, 4 h) on spontaneous locomotor activity in the rat. *: significantly different from vehicle controls.

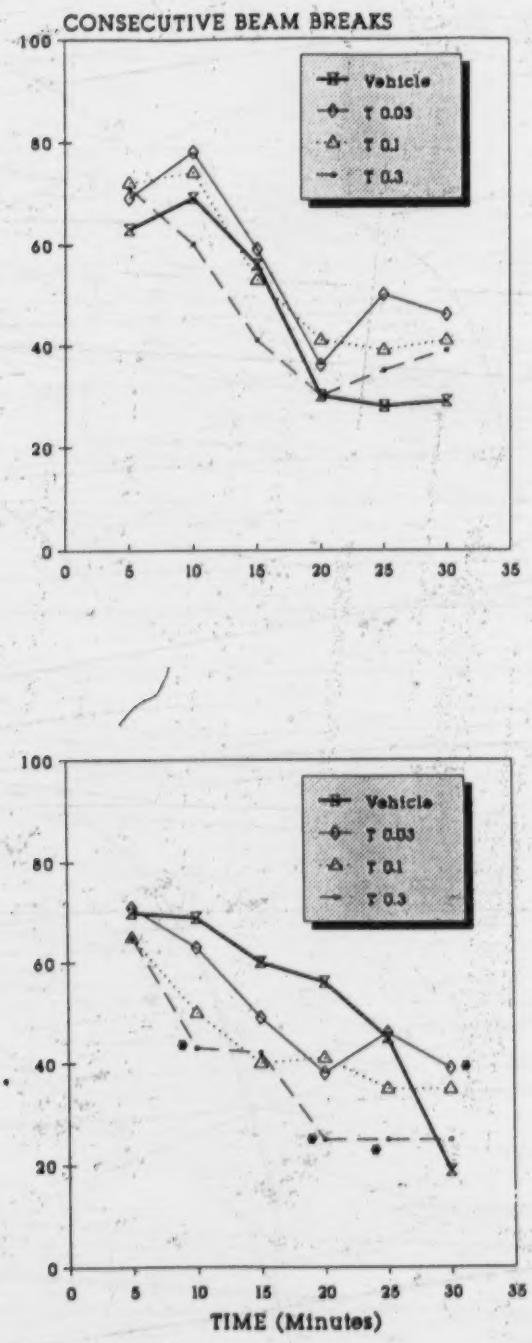


Figure 22: Effect of T administration (in mmol kg^{-1} *sc*, 0 min) either alone (top) or in combination (bottom) with clorgyline (1 mg kg^{-1} *sc*, 4 h) on locomotor activity in the rat. *: significantly different from vehicle controls.

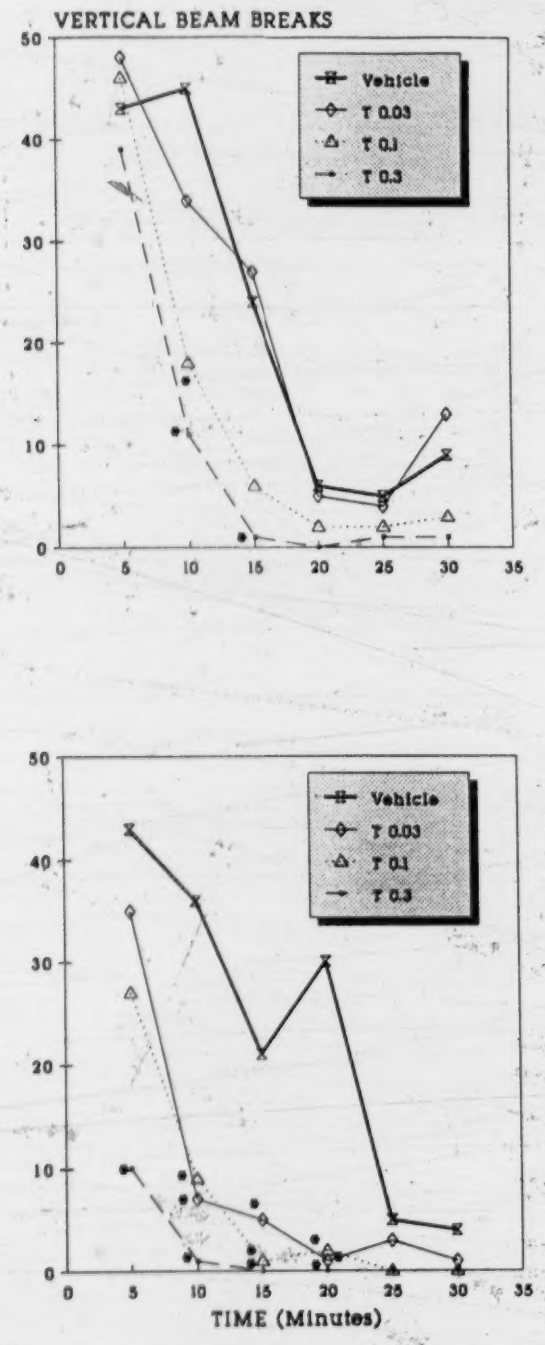


Figure 23: Effect of T administration (in mmol kg^{-1} *sc*, 0 min) either alone (top) or in combination (bottom) with clorgyline (1 mg kg^{-1} *sc*, 4 h) on locomotor activity in the rat. *: significantly different from vehicle controls.

(Figure 22). The effect of clorgyline pretreatment in this activity category, although not significant as revealed by the ANOVA [clorgyline x doses, $F(3,72)=3.09$], altered the locomotor activity in the vehicle-treated rats enough, such that *post hoc* analysis revealed significant dose-dependent effects only in the clorgyline pretreated rats (Figure 22, bottom). Vertical (rearing) activity, was decreased in a dose- [$F(3,72)=21.12$] and time-dependent [$F(5,360)=65.17$] manner (Figure 23). Clorgyline pretreatment did not significantly affect the T-induced locomotor suppressant effect [clorgyline x doses, $F(3,72)=3.40$] in this activity category.

4.E.2 Acute administration of T, either alone or in combination with clomipramine: Locomotor effects

Rats having received a pretreatment injection of clomipramine (10 mg kg^{-1} *sc*, 15 min) followed by an injection of T (0.03, 0.1 or 0.3 mmol kg^{-1} *sc*, 0 min) were placed in locomotor activity cages (see section 3.A.4 and 3.D.10) for a 30 min test period.

Total locomotor activity was decreased in a dose- [$F(3,73)=4.19$] and time-dependent [$F(5,360)=277.16$] manner. The effect of clomipramine pretreatment was such that a decrease in locomotor activity in the clomipramine pretreated control group masked the dose-dependent effects of T administration (Figure 24). A dose- [$F(3,72)=5.64$] and time-dependent [$F(5,360)=80.69$] locomotor suppressant effect was also observed in the consecutive beam break category. Clomipramine pretreatment again resulted in a loss of an evident dose-dependent locomotor suppression as a consequence of decreased locomotor activity in the vehicle treated rats (Figure 25). Vertical (rearing) activity was suppressed in a dose- [$F(3,72)=22.03$] and time-dependent [$F(5,360)=155.59$] manner. Although the ANOVA revealed a significant interaction term [clomipramine x doses, $F(3,72)=7.43$], *post hoc* analysis did not reveal any significant potentiation of the T-induced locomotor suppression (*n.b.* a trend in clomipramine potentiation of the T-induced suppression was observed in the low dose T-treated group) (Figure 26).

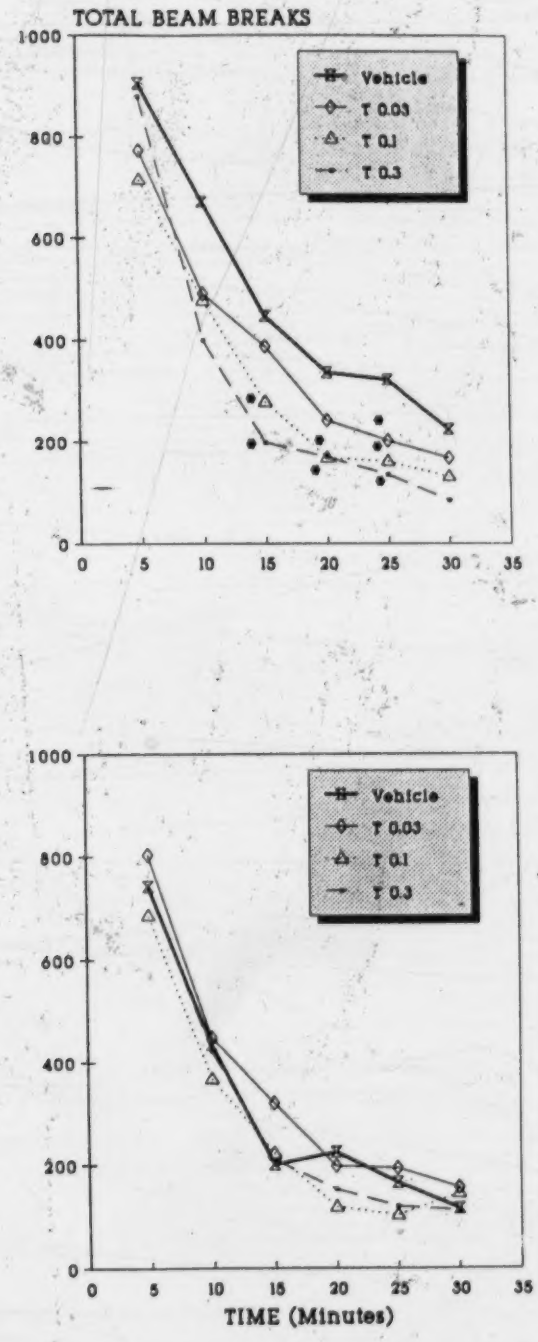


Figure 24: Effect of T administration (in mmol kg^{-1} *sc*, 0 min) either alone (top) or in combination (bottom) with clomipramine (10 mg kg^{-1} *sc*, 15 min) on spontaneous locomotor activity in the rat. *: significantly different from vehicle controls.

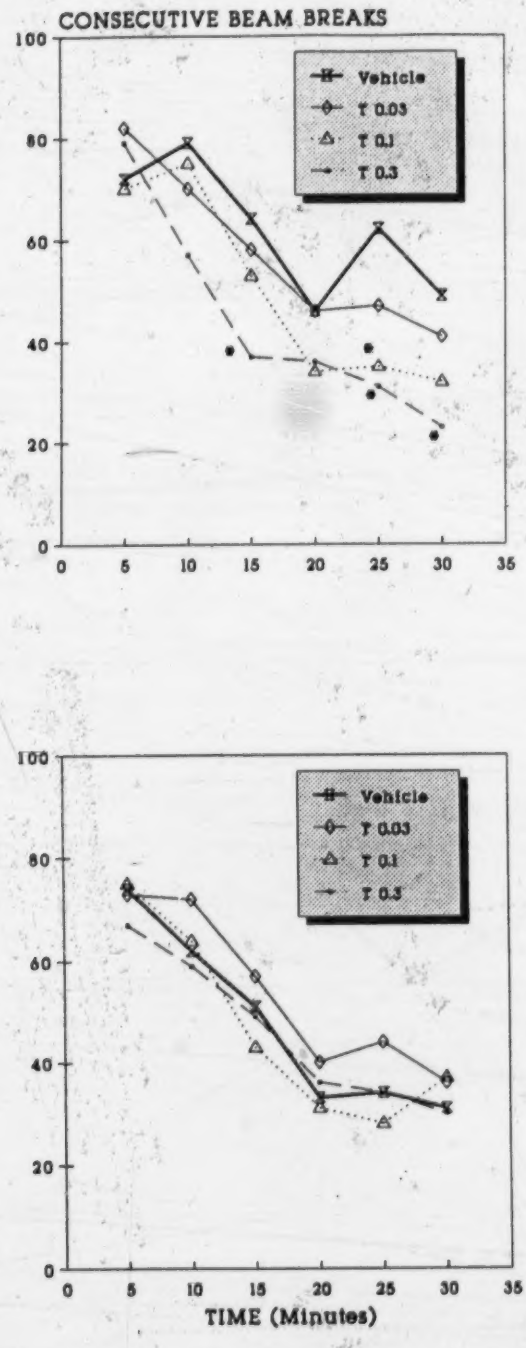


Figure 25: Effect of T administration (in mmol kg^{-1} *sc*, 0 min) either alone (top) or in combination (bottom) with clomipramine (10 mg kg^{-1} *sc*, 15 min) on locomotor activity in the rat. *: significantly different from vehicle controls.

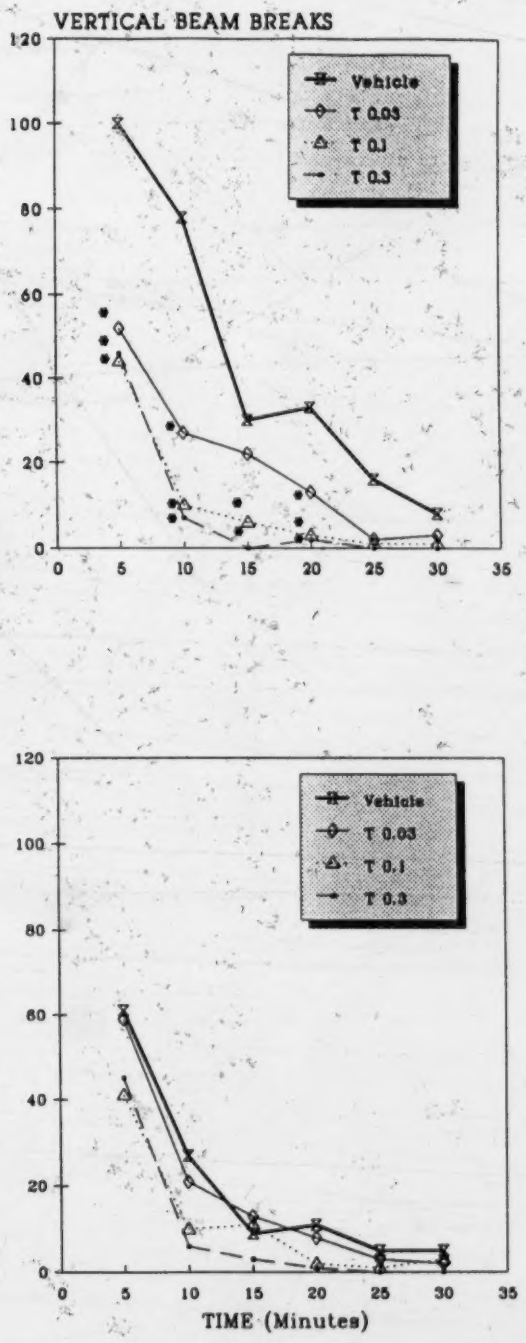


Figure 26: Effect of T administration (in mmol kg^{-1} *sc*, 0 min) either alone (top) or in combination (bottom) with clomipramine (10 mg kg^{-1} *sc*, 15 min) on locomotor activity in the rat. *: significantly different from vehicle controls.

4.E.3 Acute administration of T, either alone or in combination with (-)-deprenyl: Locomotor effects

Rats having received a pretreatment injection of (-)-deprenyl (1 mg kg^{-1} *sc*, 2 h) followed by an injection of T (0.03, 0.1 or 0.3 mmol kg^{-1} *sc*, 0 min) were placed in locomotor activity cages for a 1 h test period. MAO inhibition levels were also measured to confirm the efficacy of (-)-deprenyl administration.

The results of this experiment are depicted in Figure 27. A time-dependent motor effect was observed in the total activity category [F(5,200)=266.45], and was shown to be an interaction (treatment x time) resulting in both a motor suppressant (at 10 and 20 min) and motor excititatory (at 50 min) effect [F(20,200)=4.41]. Consecutive activity was also affected by time [F(5,200)=91.45] and was (treatment x time; F(20,200)=3.01) both suppressed (at 20 min) and increased (at 50 min) over time. Vertical activity was suppressed by treatment [F(4,40)=11.49] over time [F(5,200)=346.70]. An interaction (treatment x time; F(20,200)=5.86) was also observed. The levels of MAO inhibition following (-)-deprenyl treament demonstrate this drug's selective MAO-B inhibiting capacity. The extent of MAO inhibition was measured only in vehicle- and (-)-deprenyl (alone)-treated animals. MAO-A was inhibited by 17% [F(1,18)=46.20] whereas MAO-B was inhibited by 70% [F(1,18)=1734.44] (Table 18).

4.E.4 Acute administration of the T prodrug N-2-cyanoethyltryptamine (CET), either alone or in combination with clorgyline: Locomotor effects

Rats having received a pretreatment injection of clorgyline (1 mg kg^{-1} *sc*, 4 h) followed by an injection of CET (0.03, 0.1 or 0.3 mmol kg^{-1} *sc*, 0 min) were placed in locomotor activity cages for a 30 min test period.

Total locomotor activity was decreased in a dose- [F(3,72)=15.70] and time-dependent [F(5,360)=121.25]. Clorgyline pretreatment did not potentiate the dose-dependent effect

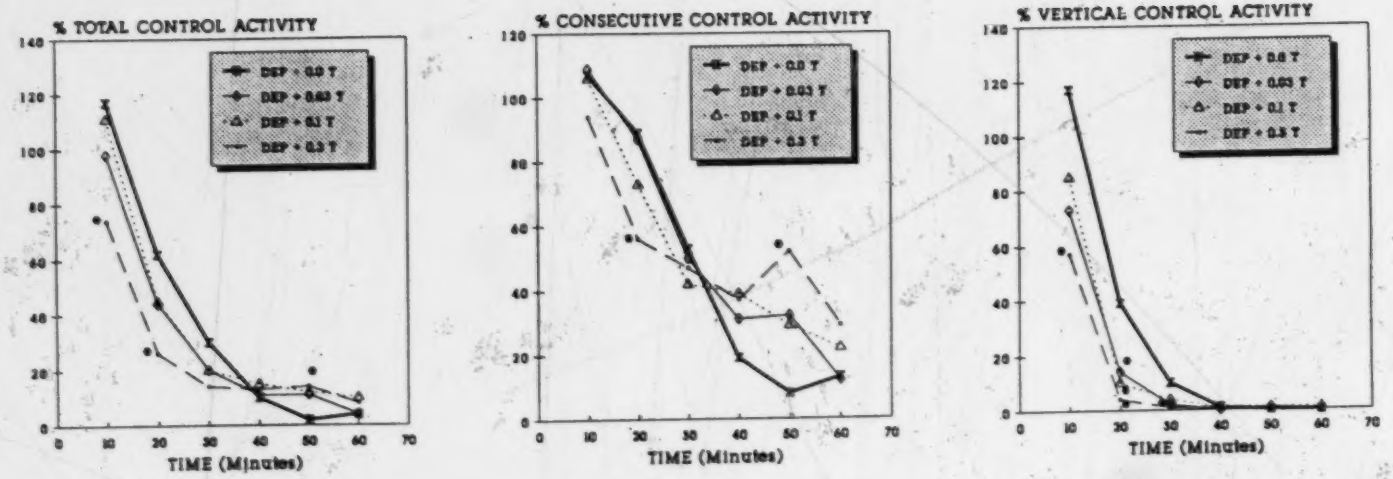


Figure 27: Effect of T administration (in mmol kg^{-1} *sc*, 0 min) in combination with DEP (1 mg kg^{-1} *sc*, 2 h) on spontaneous locomotor activity in the rat. *: significantly different from DEP controls.

	MAO Inhibition (%)	
Treatment	MAO-A	MAO-B
(-)-Deprenyl	17 (2)*	70 (1)*

Table 18: Level of MAO inhibition achieved in brain tissue following acute administration of (-)-deprenyl (1 mg kg^{-1} *sc*, 2 h). Values represent % inhibition relative to vehicle-treated controls. Mean ± (SEM); *: significantly different from control levels.

[clorgyline x dose, F(3,72)=1.49]. In fact this dose-dependent locomotor suppressant effect was lost following clorgyline pretreatment as a consequence of a clorgyline-induced decrease in locomotor activity in the control group (Figure 28). A dose- [F(3,72)=20.73] and time-dependent [F(5,360)=56.20] decrease in consecutive locomotor activity was also observed (Figure 29). As with total activity, the effect of doses of CET was lost following clorgyline pretreatment [F(3,72)=3.43] as a result of a decrease in locomotor activity in the control group. A similar trend in effects of CET on vertical activity was observed with a dose- [F(3,72)=15.56] and time-dependent [F(5,360)=51.09] suppression of locomotor activity, the former of which was lost following clorgyline pretreatment [F(3,72)=1.49] (Figure 30).

4.E.5 Acute administration of the T prodrug CET, either alone or in combination with clomipramine: Locomotor effects

Rats having received a pretreatment injection of clomipramine (10 mg kg^{-1} *sc*, 15 min) followed by an injection of CET (0.03, 0.1 or 0.3 mmol kg^{-1} *sc*, 0 min) were placed in locomotor activity cages for a 30 min test period.

Total locomotor activity was decreased in a dose- [F(3,72)=26.72] and time-dependent [F(5,360)=298.16] manner. This dose-dependent locomotor suppression was potentiated by clomipramine pretreatment [clomipramine x doses, F(3,72)=9.54] (Figure 31). A dose- [F(3,72)=29.64] and time-dependent [F(5,360)=81.01] suppressant effect was also observed in the consecutive activity category. This was not potentiated following clomipramine pretreatment [clomipramine x doses, F(3,72)=1.29] (Figure 32). Vertical activity was decreased in a dose- [F(3,72)=43.53] and time-dependent [F(5,360)=136.25]. The dose-dependent effect was potentiated by clomipramine pretreatment [clomipramine x doses, F(3,72)=13.44] (Figure 33).

Discussion: Locomotor activity following T administration has often been reported to result in locomotor stimulant or behavioural excitation (Dewhurst and Marley, 1965; Foldes and

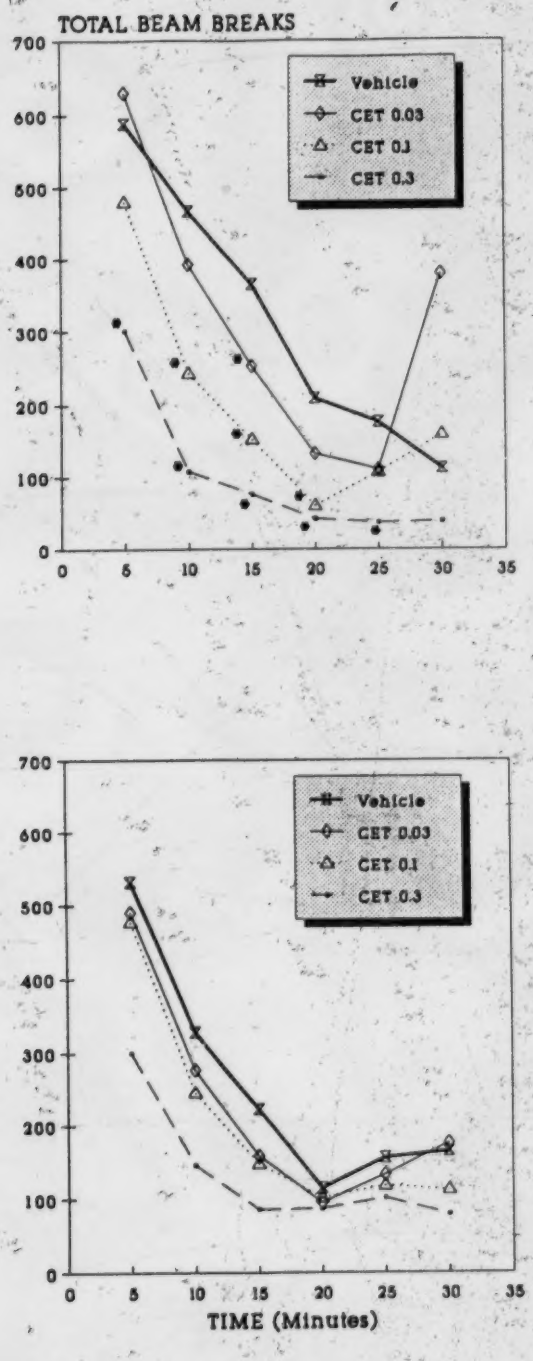


Figure 28: Effect of CET administration (in mmol kg^{-1} *sc*, 0 min) either alone (top) or in combination (bottom) with clorgyline (1 mg kg^{-1} *sc*, 4 h) on spontaneous locomotor activity in the rat. *: significantly different from vehicle controls.

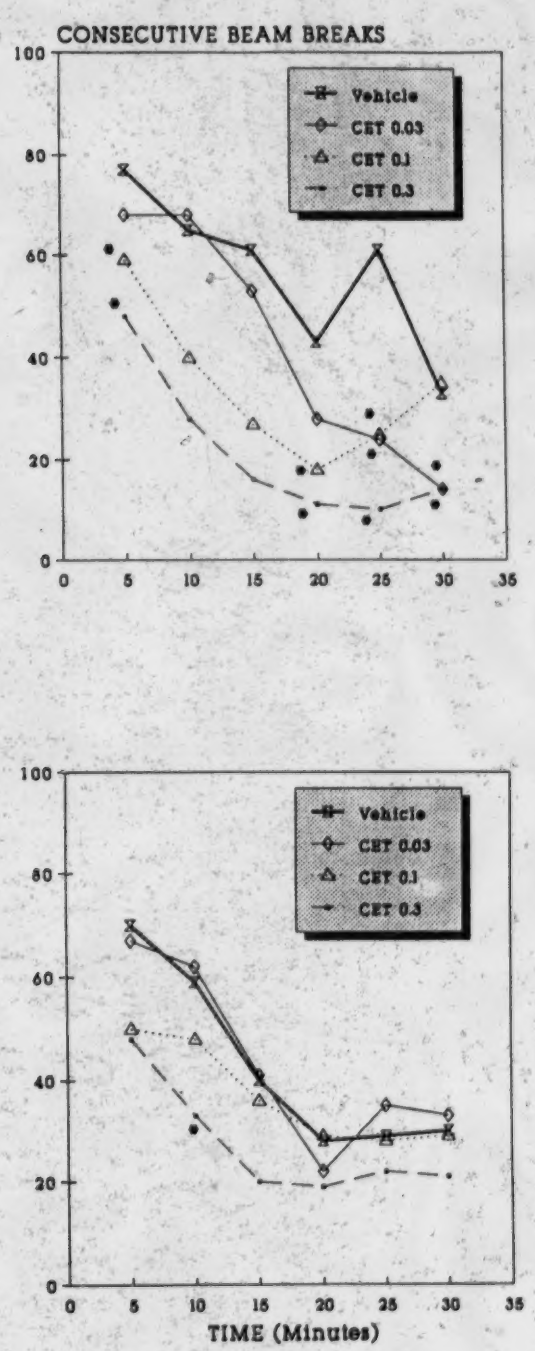


Figure 29: Effect of CET administration (in mmol kg^{-1} *sc*, 0 min) either alone (top) or in combination (bottom) with clorgyline (1 mg kg^{-1} *sc*, 4 h) on locomotor activity in the rat. *: significantly different from vehicle controls.

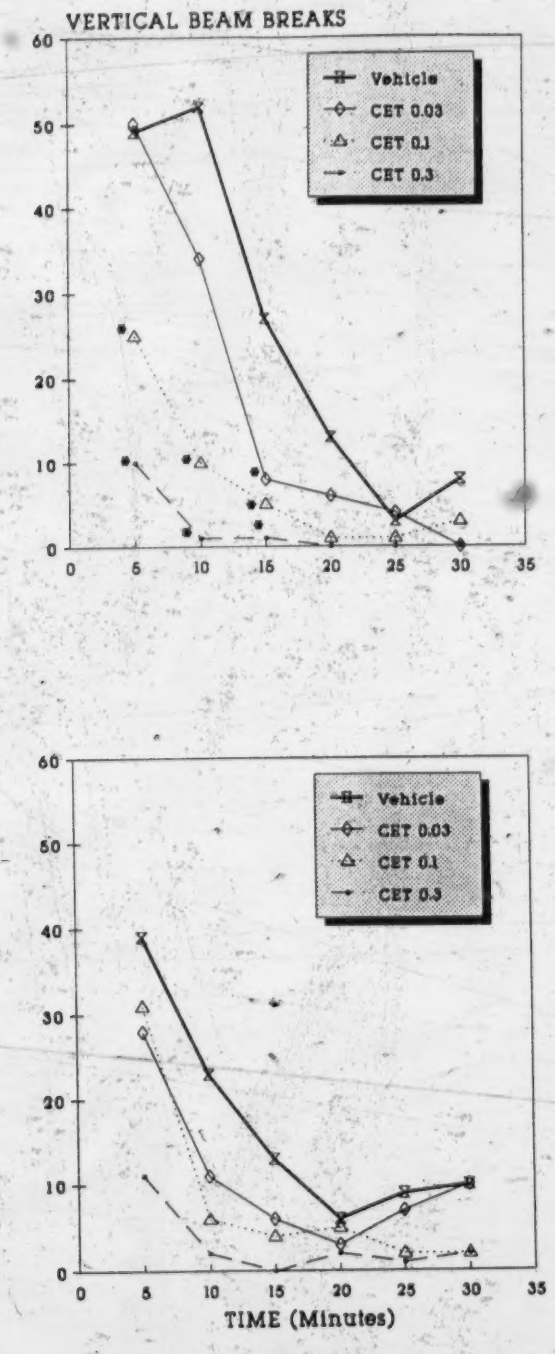


Figure 30: Effect of CET administration (in mmol kg^{-1} *sc*, 0 min) either alone (top) or in combination (bottom) with clorgyline (1 mg kg^{-1} *sc*, 4 h) on locomotor activity in the rat. *: significantly different from vehicle controls.

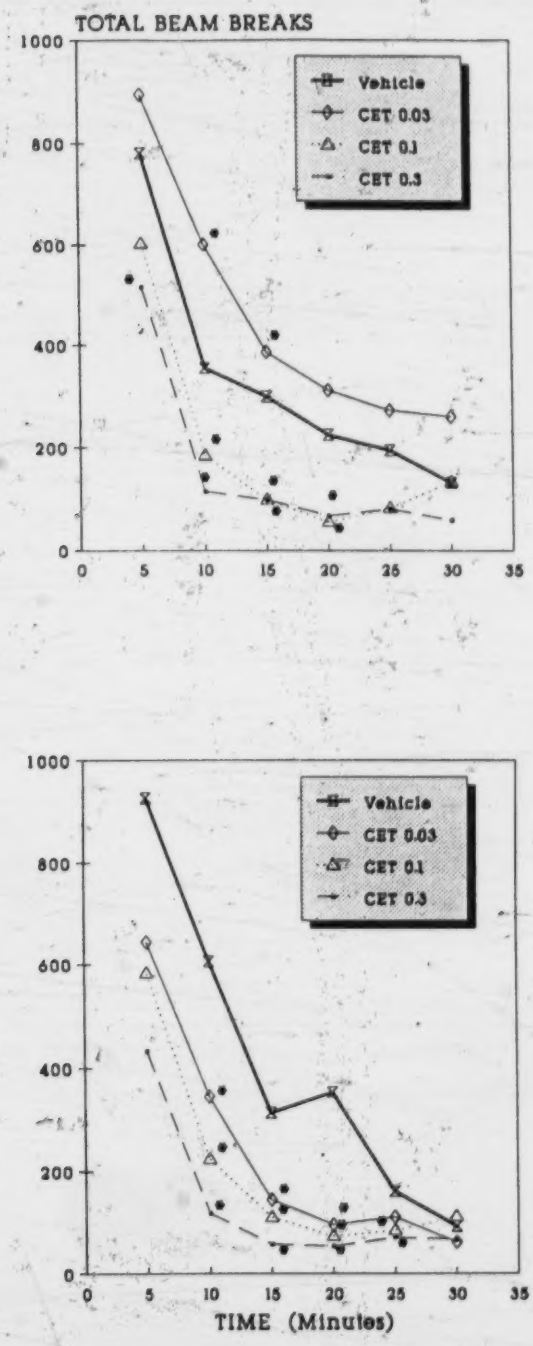


Figure 31: Effect of CET administration (in mmol kg^{-1} *sc*, 0 min) either alone (top) or in combination (bottom) with clomipramine (10 mg kg^{-1} *sc*, 15 min) on spontaneous locomotor activity in the rat. *: significantly different from vehicle controls.

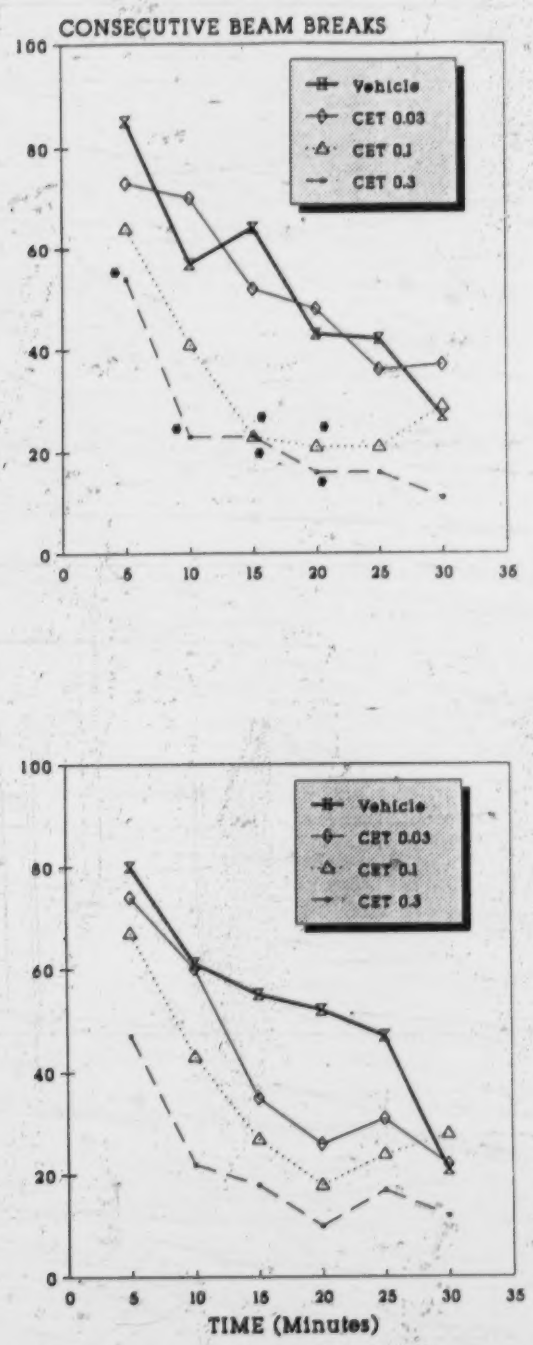


Figure 32: Effect of CET administration (in mmol kg^{-1} *sc*, 0 min) either alone (top) or in combination (bottom) with clomipramine (10 mg kg^{-1} *sc*, 15 min) on locomotor activity in the rat. *: significantly different from vehicle controls.

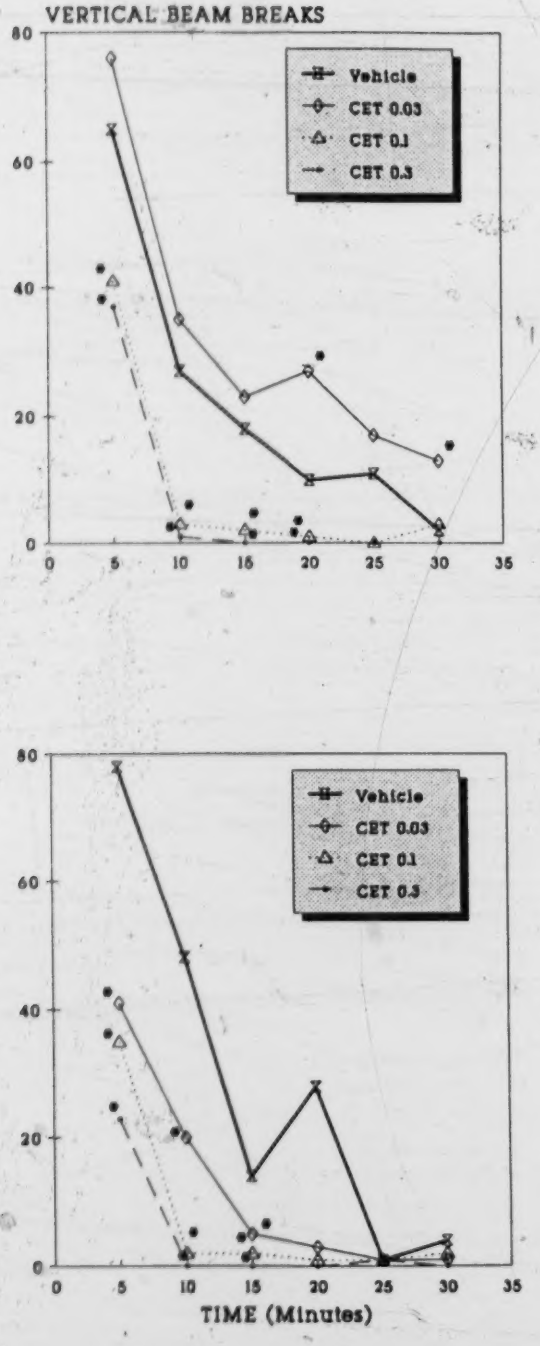


Figure 33: Effect of CET administration (in mmol kg^{-1} *sc*, 0 min) either alone (top) or in combination (bottom) with clomipramine (10 mg kg^{-1} *sc*, 15 min) on locomotor activity in the rat. *: significantly different from vehicle controls.

Costa, 1975; Marsden and Curzon, 1978). It is possible that the route of administration was an important factor in these previous studies as T was administered by *icv* injection, and thus it may be argued that T was not subject to extensive hepatic metabolism or other possible catabolic routes. However, studies by Quock and Weick (1978) and Tedeschi *et al.* (1959), in which T was administered peripherally, also demonstrated motor excitation. The pretreatment with MAO inhibitors in these latter experiments potentiated the excitatory effects of T. The observation in this present series of experiments that T induces a locomotor suppressant effect is not entirely in contradiction to previous findings. Ernst *et al.* (1961) and Saavedra *et al.* (1970) also observed a reduction in spontaneous locomotor activity. This latter effect was not potentiated by MAO inhibition. It has been argued, however, that because the reduction in locomotor activity was accompanied by tremor and rigidity, this might be a manifestation of central excitatory effects (Jones, 1982d). The motor suppressant effects of T were potentiated by clorgyline pretreatment in the present study. As a clorgyline treatment does not specifically increase levels of T, but rather affects many of the biogenic amines, it is a distinct possibility that the effects of T on locomotor activity or behaviour are a result of an interaction with one or more of the other neurotransmitter systems. Marsden and Curzon (1978) proposed this hypothesis following the observation that depletion of 5-HT with *p*CPA reduced the behavioural effects of exogenous T. These same authors noticed that the depletion of endogenous T, but not 5-HT, following AAD inhibition, attenuated the 5-HT syndrome (see section 2.B.2) induced by tryptophan (Marsden and Curzon, 1979).

The structural similarity between T and 5-HT led to the investigation of potential interactions between exogenous T and the 5-HT uptake inhibitor clomipramine. Although clomipramine potentiated the T-induced suppression of vertical locomotor activity, the lack of a consistent effect of clomipramine on T-induced changes in locomotor activity is an indication that T may not exert its effects primarily through the 5-HT system. Clomipramine is demethylated

to the secondary amine desmethylclomipramine (Flament *et al.*, 1987; Benfield *et al.*, 1980). This active metabolite is known to affect catecholamine systems (Randrup and Braestrup, 1977). It is possible that the changes in vertical activity elicited by the combination of T and clomipramine are indicative of an action of T on a catecholamine system, particularly the DA system, perhaps resulting in central excitatory effects (Jones, 1982d). The excitatory effects of T observed by Tedeschi *et al.* (1959) were shown to be antagonized by relatively high doses of neuroleptics, which may be attributable to the actions of these compounds on DA receptors.

The potentiation of T-induced suppression of locomotion following (-)-deprenyl pretreatment indicates that, functionally, T is a mixed substrate for MAO. As MAO-A activity was only inhibited by 17% (see Section 4.E.3), it would seem that the extent of MAO-B inhibition was sufficient to protect T from catabolism. This effect had previously been shown in this series of experiments by the reduction in the density of [^{3}H]-T binding sites following chronic (-)-deprenyl treatment (see Section 4.B.2). The appearance of a motor stimulant effect following acute T administration has been shown previously (Tedeschi *et al.*, 1959; Dewhurst and Marley, 1965; Quock and Weick, 1978; Marsden and Curzon, 1978, 1979). This motor excitation has been attributed to an enhancement of 5-HT-mediated behaviours as well as to other central (possibly DA-mediated) stimulatory effects (see Jones, 1982d). The comparison of the behavioural effects of T and CET yielded contrasting results. It was hypothesized that if CET was an effective prodrug of T, then its effect on locomotor activity would be similar to that observed with T. This was, however, not the case as, contrary to the clorgyline potentiation of T-induced suppression of locomotion, CET-induced locomotor suppression was potentiated by clomipramine treatment. This might well indicate an action of clomipramine on the 5-HT system as well as an action on the DA system (via a metabolite of clomipramine, see above).

4.E.6 Acute administration of T and the prodrug CET, either alone or in combination with clorgyline or clomipramine: Neurochemical effects

Introduction: As the midrange dose (i.e. 0.1 mmol kg^{-1}) of both T and CET, in combination with either or both pretreatments [i.e. clorgyline (1 mg kg^{-1} *sc*, 4 h) or clomipramine (10 mg kg^{-1} *sc*, 15 min)], was demonstrated as being an effective motor suppressant dose, inducing significant effects at 15 min post-injection (Figures 21 to 26 and 28 to 33), it was decided to use this dose- and time-course as the treatment for biogenic amine analyses using GC (see Section 2.A.2) and HPLC-ECD procedures.

Results:

4.E.6.1 Brain and liver T and 5-HT levels and MAO activity

Brain: Levels of T were significantly increased in the brain tissue of rats having received CET [$F(2,15)=4.79$] or clorgyline [$F(2,30)=8,03$] (Table 19). Brain 5-HT levels were significantly increased following CET [$F(2,15)=41.43$]. This effect was evident in the vehicle- or clomipramine-pretreated animal tissue (Table 20). Clorgyline significantly increased 5-HT levels [$F(2,30)=514.62$]. An interaction between clorgyline and CET was also found [$F(4,30)=15.21$].

MAO activity was also investigated in this tissue. Clorgyline preferentially inhibited MAO-A activity [$F(2,30)=2396.01$]. Neither T or CET treatments affected brain MAO activity (Table 21). A similar effect was observed with MAO-B activity. Only clorgyline exhibited any significant inhibitory effect [$F(2,30)=51.03$], although not to the same extent as seen with MAO-A.

Liver: In this tissue only CET treatment resulted in significant increases in T levels [$F(2,15)=131.54$] (Table 19). Liver 5-HT levels were increased significantly following either CET [$F(2,15)=79.14$] or clorgyline [$F(2,30)=3.77$] treatments (Table 20). No interaction between these two compounds was evident [$F(4,30)=0.07$].

BRAIN	Vehicle	T	CET
Vehicle	n.d.	n.d.	16 (5)^{*,a}
CLG	n.d.	15 (3)^{a,c,g}	17 (5)^{b}
CMI	n.d.	n.d.	9 (1)^{f,g}

LIVER	Vehicle	T	CET
Vehicle	n.d.	n.d.	94 (12)^{*,a}
CLG	n.d.	n.d.	94 (17)^{b,e}
CMI	n.d.	12 (5)^{a,f}	91 (11)^{f,g}

Table 19: Effect of clorgyline (CLG; 1 mg kg^{-1} *sc*, 15 min) and clomipramine (CMI; 10 mg kg^{-1} *sc*, 4 h) pretreatment on whole brain [top] and liver [bottom] T levels following acute administration of T or CET (each at 0.1 mmol kg^{-1} *sc*, 15 min). Mean (ng g^{-1} wet weight tissue) ± (SEM); n.d.: not detected (i.e. <1 ng g^{-1}); Superscripts represent significant differences within treatment regimens = *: *vs* veh/veh; a: *vs* veh/T; b: *vs* veh/CET; c: *vs* CLG/veh; d: *vs* CLG/T; e: *vs* CLG/CET; f: *vs* CMI/veh; g: *vs* CMI/T; h *vs* CMI/CET.

BRAIN	Vehicle	T	CET
Vehicle	295 (18)	332 (18)	649 (42)^{e,a}
CLG_3	1006 (30)^{e,f}	1067 (25)^{a,a}	1035 (24)^{b,b}
CMI	369 (17)	372 (35)	626 (31)^{f,a}

LIVER	Vehicle	T	CET
Vehicle	60 (6)	53 (5)	223 (25)^{e,a}
CLG	118 (16)^{e,f}	104 (15)^{a,a}	256 (38)^{c,d}
CMI	62 (5)	58 (7)	217 (43)^{f,a}

Table 20: Effect of clorgyline (CLG; 1 mg kg^{-1} *sc*, 15 min) and clomipramine (CMI; 10 mg kg^{-1} *sc*, 4 h) pretreatment on whole brain [top] and liver [bottom] 5-HT levels following acute administration of T or CET (each at 0.1 mmol kg^{-1} *sc*, 15 min). Mean (ng g^{-1} wet weight tissue) ± (SEM); See Table 19 for definitions of superscripts.

MAO-A	Vehicle	T	CET
Vehicle	0 (1)	1 (1)	0 (3)
CLG	97 (1)^{e,f}	97 (1)^{a,g}	98 (0)^{b,h}
CMI	2 (2)	1 (3)	2 (3)

MAO-B	Vehicle	T	CET
Vehicle	0 (2)	7 (3)	0 (5)
CLG	32 (7)^{e,f}	30 (1)^{a,g}	29 (2)^{b,h}
CMI	5 (2)	5 (2)	10 (5)

Table 21: Effect of clorgyline (CLG; 1 mg kg^{-1} *sc*, 15 min) and clomipramine (CMI; 10 mg kg^{-1} *sc*, 4 h) pretreatment on inhibition of brain MAO activity following acute administration of T or CET (each at 0.1 mmol kg^{-1} *sc*, 15 min). Mean ± (SEM); See Table 19 for definitions of superscripts.

MAO-A	Vehicle	T	CET
Vehicle	10 (4)	2 (2)	4 (4)
CLG	24 (6)	33 (8)[a]	27 (6)[c]
CMI	7 (7)	0 (0)	1 (1)

MAO-B	Vehicle	T	CET
Vehicle	11 (7)	7 (6)	1 (1)
CLG	2 (2)	0 (1)	2 (1)
CMI	1 (1)	5 (5)	1 (1)

Table 22: Effect of clorgyline (CLG; 1 mg kg^{-1} *sc*, 15 min) and clomipramine (CMI; 10 mg kg^{-1} *sc*, 4 h) pretreatment on inhibition of liver MAO activity following acute administration of T or CET (each at 0.1 mmol kg^{-1} *sc*, 15 min). Mean ± (SEM). See Table 19 for definitions of superscripts.

Clorgyline preferentially inhibited MAO-A activity [F(2,30)=21.72], and did not have any inhibitory effect on MAO-B activity [F(2,30)=0.60]. Neither T nor CET inhibited MAO activity [F(2,15)=0.30 and F(2,15)=1.56 for MAO-A and -B, respectively] (Table 22).

4.E.6.2 Acute administration of T or CET and/or clorgyline or clomipramine: Brain levels of biogenic amines and selected acid metabolites

Equimolar doses of T or CET (0.1 mmol kg^{-1} *sc*, 15 min) were administered to rats. Clorgyline (1 mg kg^{-1} *sc*, 4 h) or clomipramine (10 mg kg^{-1} *sc*, 15 min) were used as pretreatments as stated in section 4.E.6.1. The FC, HYPO, HIPPO, CN and olfactory tubercle (OT) were assayed for DA, 5-HT and their respective acid metabolites, DOPAC and HVA, and 5-HIAA using HPLC-ECD. The clorgyline and clomipramine pretreatments will be referred to as "antidepressant" (AD), and the T and CET treatments will be referred to as T/CET in this following section for clarity.

Frontal Cortex

AD increased DA [F(2,30)=8.39] and 5-HT [F(2,30)=136.95] levels while decreasing DOPAC [F(2,30)=5.38], HVA [F(2,30)=11.49] and 5-HIAA [F(2,30)=137.84] levels. T/CET treatment resulted in increased 5-HT [F(2,15)=20.35] and decreased DOPAC [F(2,15)=5.06] and 5-HIAA [F(2,15)=56.36] levels. An interaction between AD and T/CET [F(4,30)21.34] was observed (Tables 23 and 24).

Hypothalamus

Levels of DA [F(2,30)=6.78], 5-HT [F(2,30)=322.70], 5-HIAA [F(2,30)=93.76] and DOPAC [F(2,30)=28.76] were altered following AD pretreatment. 5-HT levels [F(2,15)=30.04] were increased with T/CET whereas a reduction in the levels of DOPAC [F(2,15)=17.97] and 5-HIAA [F(2,15)=56.05] occurred following T/CET. Interactions between AD and T/CET affected DOPAC [F(4,30)=7.09], HVA [F(4,30)=2.84], 5-HT [F(4,30)=16.76] and 5-HIAA [F(4,30)=14.45] levels (Tables 25 and 26).

FRONTAL CORTEX

DA	Vehicle	T	CET
Vehicle	27 (6)	33 (5)	39 (4)
CLG	46 (5)*	55 (5)	45 (6)
CMI	37 (9)	33 (5)	46 (3)

DOPAC	Vehicle	T	CET
Vehicle	56 (15)	79 (17)	26 (5)[a]
CLG	30 (11)	30 (8)[a]	19 (5)
CMI	70 (14)	70 (24)	37 (8)

HVA	Vehicle	T	CET
Vehicle	54 (9)	38 (4)	48 (9)
CLG	16 (5)*,[f]	16 (8)	3 (3)[b]
CMI	66 (17)	50 (21)	34 (17)

Table 23: Effect of combined CLG (1 mg kg^{-1} *sc*, 15 min) or CMI (10 mg kg^{-1} *sc*, 4 h) pretreatment and T or CET (each at 0.1 mmol kg^{-1} *sc*, 15 min) treatment on frontal cortex DA, DOPAC and HVA content. Mean (ng g^{-1} wet weight tissue) ± (SEM). See Table 19 for definitions of superscripts.

FRONTAL CORTEX

5-HT	Vehicle	T	CET
Vehicle	642 (31)	632 (46)	1175 (57)^{*,a}
CLG	1790 (146)^{*,f}	1748 (111)^{a,a}	1930 (83)^{b,b}
CMI	660 (48)	664 (26)	1173 (133)^{f,a}

5-HIAA	Vehicle	T	CET
Vehicle	365 (28)	339 (24)	124 (8)^{*,a}
CLG	80 (9)^{*,f}	71 (5)^{a,a}	81 (3)^{b,b}
CMI	321 (26)	299 (16)	116 (9)^{f,a}

Table 24: Effect of combined CLG (1 mg kg^{-1} *sc*, 15 min) or CMI (10 mg kg^{-1} *sc*, 4 h) pretreatment and T or CET (each at 0.1 mmol kg^{-1} *sc*, 15 min) treatment on frontal cortex 5-HT and 5-HIAA content. Mean (ng g^{-1} wet weight tissue) ± (SEM). See Table 19 for definitions of superscripts.

HYPOTHALAMUS

DA	Vehicle	T	CET
Vehicle	174 (11)	192 (18)	283 (55)
CLG	324 (27)$^{*,\delta}$	427 (89)$^{\alpha}$	306 (22)
CMI	197 (10)	273 (68)	278 (45)

DOPAC	Vehicle	T	CET
Vehicle	157 (15)	152 (16)	76 (5)$^{*,\alpha}$
CLG	48 (10)$^{*,\delta}$	71 (6)$^{\beta}$	51 (4)
CMI	212 (10)*	238 (49)	53 (12)$^{\delta,\alpha}$

HVA	Vehicle	T	CET
Vehicle	26 (15)	12 (8)	10 (7)
CLG	10 (5)	9 (7)	32 (17)
CMI	37 (8)	41 (16)	3 (3)$^{\delta}$

Table 25: Effect of combined CLG (1 mg kg^{-1} *sc*, 15 min) or CMI (10 mg kg^{-1} *sc*, 4 h) pretreatment and T or CET (each at 0.1 mmol kg^{-1} *sc*, 15 min) treatment on hypothalamus DA, DOPAC and HVA content. Mean (ng g^{-1} wet weight tissue) ± (SEM). See Table 19 for definitions of superscripts.

HYPOTHALAMUS

5-HT	Vehicle	T	CET
Vehicle	487 (93)	692 (51)*	1693 (50)^{*,a}
CLG	2221 (125)^{*,f}	2634 (109)^{a,g}	2466 (152)^{b,h}
CMI	792 (79)*	706 (50)	1452 (83)^{f,g}

5-HIAA	Vehicle	T	CET
Vehicle	488 (69)	565 (17)	275 (19)^{*,a}
CLG	148 (12)^{*,f}	150 (10)^{a,g}	159 (24)^{b,h}
CMI	665 (30)*	593 (35)	229 (26)^{f,g}

Table 26: Effect of combined CLG (1 mg kg^{-1} *sc*, 15 min) or CMI (10 mg kg^{-1} *sc*, 4 h) pretreatment and T or CET (each at 0.1 mmol kg^{-1} *sc*, 15 min) treatment on hypothalamus 5-HT and 5-HIAA content. Mean (ng g^{-1} wet weight tissue) ± (SEM). See Table 19 for definitions of superscripts.

Hippocampus

AD pretreatment induced a decrease in DOPAC [F(2,30)=14.06] and 5-HIAA [F(2,30)=76.68] whereas 5-HT levels were increased by both AD [F(2,30)=73.98] and T/CET treatment [F(2,15)=22.04]. 5-HIAA levels were reduced by T/CET [F(2,15)=12.38]. An interaction between pretreatments and treatments was observed for DA [F(4,30)=3.73], 5-HT [F(4,30)=4.66] and 5-HIAA [F(4,30)=4.59] (Tables 27 and 28).

Striatum

The effects of AD pretreatment was again evident in this region as alterations in the levels of all of the measured compounds; DA [F(2,30)=70.37], DOPAC [F(2,30)=43.30], HVA [F(2,30)=175.27], 5-HT [F(2,30)=76.75 and 5-HIAA [F(2,30)=81.26]. T/CET treatment also elicited potent effects on DOPAC [F92,15)=17.14], HVA [F(2,15)=17.24], 5-HT [F(2,15)=12.90] and 5-HIAA [F(2,15)=11.78]. The levels of DOPAC [F(4,30)=6.75], HVA [F(4,30)=4.00] and 5-HIAA [F(4,30)=2.90] were also affected by the combination of AD and T/CET treatments (Tables 29 and 30).

Olfactory Tubercle

The measured levels of both DA and 5-HT as well as their respective acid metabolites were all independently affected by AD and T/CET treatments. AD: DA [F(2,30)=65.17]; DOPAC [F(2,30)=65.40]; HVA [F(2,30)=52.34; 5-HT [F(2,30)=167.06]; 5-HIAA [F(2,30)=143.48]. T/CET: DA [F(2,15)=5.69]; DOPAC [F(2,15)=48.96]; HVA [F(2,15)=9.56]; 5-HT [F(2,15)=39.43]; 5-HIAA [F(2,15)=31.08]. Interactions between AD and T/CET were also evident for DOPAC [F(4,30)=8.92], 5-HT [F(4,30)=4.37 and 5-HIAA [F(4,30)=11.85] (Tables 31 and 32).

Discussion: The administration of T altered amine metabolism in a manner consistent with previous findings. T is known to exist in areas of the brain rich in DA (Altar *et al.*, 1986) and levels of this trace amine have been shown, either through direct GC-MS assaying techniques or via binding parameter assessment, to respond to lesions of brain DA systems

HIPPOCAMPUS

DA	Vehicle	T	CET
Vehicle	7 (1)	6 (2)	11 (2)
CLG	10 (2)	12 (3)	7 (2)
CMI	2 (2)	7 (0)	13 (3)

DOPAC	Vehicle	T	CET
Vehicle	28 (2)	27 (4)	22 (2)
CLG	14 (1)$^{*,\delta}$	18 (1)	16 (2)
CMI	26 (3)	31 (3)	21 (4)

HVA	Vehicle	T	CET
Vehicle	39 (10)	35 (5)	42 (14)
CLG	24 (9)	23 (6)	32 (12)
CMI	51 (8)	32 (7)	33 (4)

Table 27: Effect of combined CLG (1 mg kg^{-1} *sc*, 15 min) or CMI (10 mg kg^{-1} *sc*, 4 h) pretreatment and T or CET (each at 0.1 mmol kg^{-1} *sc*, 15 min) treatment on hippocampus DA, DOPAC and HVA content. Mean (ng g^{-1} wet weight tissue) ± (SEM). See Table 19 for definitions of superscripts.

HIPPOCAMPUS

5-HT	Vehicle	T	CET
Vehicle	366 (16)	443 (29)	817 (64)^{a,a}
CLG	1039 (85)^{a,f}	1082 (59)^{a,a}	1054 (86)
CMI	481 (27)	452 (34)	755 (55)^{f,a}

5-HIAA	Vehicle	T	CET
Vehicle	350 (35)	323 (17)	220 (18)^{a,a}
CLG	127 (6)^{a,f}	120 (5)^{a,a}	128 (9)^{b,b}
CMI	317 (23)	259 (18)^{f}	204 (17)^{f}

Table 28: Effect of combined CLG (1 mg kg^{-1} *sc*, 15 min) or CMI (10 mg kg^{-1} *sc*, 4 h) pretreatment and T or CET (each at 0.1 mmol kg^{-1} *sc*, 15 min) treatment on hippocampus 5-HT and 5-HIAA content. Mean (ng g^{-1} wet weight tissue) ± (SEM). See Table 19 for definitions of superscripts.

STRIATUM

DA	Vehicle	T	CET
Vehicle	8221 (588)	7264 (852)	8944 (532)
CLG	13325 (793)^{*,f}	13489 (388)^{a,g}	13893 (647)^{b,h}
CMI	8283 (268)	9096 (947)	8579 (591)

DOPAC	Vehicle	T	CET
Vehicle	2594 (214)	2267 (276)	1049 (123)^{*,a}
CLG	752 (88)^{*,f}	686 (112)^{a,g}	829 (121)
CMI	2490 (226)	2323 (299)	1146 (156)^{f,g}

HVA	Vehicle	T	CET
Vehicle	909 (65)	693 (24)*	531 (54)^{*,a}
CLG	131 (12)^{*,f}	102 (7)^{a,g}	116 (8)^{b,h}
CMI	906 (75)	820 (68)^{a}	639 (45)^{f}

Table 29: Effect of combined CLG (1 mg kg^{-1} *sc*, 15 min) or CMI (10 mg kg^{-1} *sc*, 4 h) pretreatment and T or CET (each at 0.1 mmol kg^{-1} *sc*, 15 min) treatment on striatum DA, DOPAC and HVA content. Mean (ng g^{-1} wet weight tissue) ± (SEM). See Table 19 for definitions of superscripts.

STRIATUM

5-HT	Vehicle	T	CET
Vehicle	460 (37)	463 (60)	797 (34)^{*,a}
CLG	1087 (25)^{*,f}	985 (70)^{a,a}	1220 (54)^{b,d,b}
CMI	570 (51)	470 (47)	643 (119)

5-HIAA	Vehicle	T	CET
Vehicle	329 (28)	309 (28)	270 (11)
CLG	139 (8)^{*,f}	111 (8)^{a,c,a,a}	151 (5)^{b,b}
CMI	338 (16)	256 (10)^{a,f}	250 (15)^{f}

Table 30: Effect of combined CLG (1 mg kg^{-1} *sc*, 15 min) or CMI (10 mg kg^{-1} *sc*, 4 h) pretreatment and T or CET (each at 0.1 mmol kg^{-1} *sc*, 15 min) treatment on striatum 5-HT and 5-HIAA content. Mean (ng g^{-1} wet weight tissue) ± (SEM). See Table 19 for definitions of superscripts.

OLFACTORY TUBERCLE

DA	Vehicle	T	CET
Vehicle	3164 (274)	3751 (368)	4866 (352)^{*,a}
CLG	6122 (329)^{*,f}	6202 (270)^{a,a}	6297 (369)^{b,b}
CMI	3694 (292)	3775 (247)	4723 (233)^{f,a}

DOPAC	Vehicle	T	CET
Vehicle	1669 (132)	1457 (189)	555 (60)^{*,a}
CLG	403 (91)^{*,f}	451 (91)^{a,a}	360 (32)^{b,b}
CMI	1735 (88)	1602 (134)	642 (35)^{f,a}

HVA	Vehicle	T	CET
Vehicle	577 (57)	484 (104)	277 (26)*
CLG	105 (19)^{*,f}	78 (14)^{a,a}	101 (13)^{b,b}
CMI	582 (71)	474 (31)	369 (32)^{f}

Table 31: Effect of combined CLG (1 mg kg^{-1} *sc*, 15 min) or CMI (10 mg kg^{-1} *sc*, 4 h) pretreatment and T or CET (each at 0.1 mmol kg^{-1} *sc*, 15 min) treatment on olfactory tubercle DA, DOPAC and HVA content. Mean (ng g^{-1} wet weight tissue) ± (SEM). See Table 19 for definitions of superscripts.

OLFACTORY TUBERCLE

5-HT	Vehicle	T	CET
Vehicle	1107 (78)	1114 (73)	1999 (116)^{a,a}
CLG	2630 (94)^{a,f}	2838 (159)^{a,a}	2934 (139)^{a,a}
CMI	1191 (63)	1164 (42)	1956 (119)^{f,a}

5-HIAA	Vehicle	T	CET
Vehicle	455 (26)	350 (26)^{a}	231 (21)^{a,a}
CLG	131 (8)^{a,f}	114 (13)^{a,a}	130 (14)^{a,a}
CMI	407 (20)	317 (17)^{f}	225 (16)^{f,a}

Table 32: Effect of combined CLG (1 mg kg^{-1} *sc*, 15 min) or CMI (10 mg kg^{-1} *sc*, 4 h) pretreatment and T or CET (each at 0.1 mmol kg^{-1} *sc*, 15 min) treatment on olfactory tubercle 5-HT and 5-HIAA content. Mean (ng g^{-1} wet weight tissue) ± (SEM). See Table 19 for definitions of superscripts.

(Juorio and Greenshaw, 1986; Juorio *et al.*, 1987a; van Nguyen *et al.*, 1989). In view of these findings the action of exogenous T on DA metabolism is not surprising. The decrease in the potassium stimulated release of [^{3}H]-DA from preloaded rat caudate nucleus slices following incubation with T (Ennis *et al.*, 1981) as well as the slight, yet significant, increase in the release of [^{3}H]-DA from striatal prisms following incubation in CSF-Ringer's solution containing T (Baker and Yasensky, 1981) indicate a neurochemical relationship between these two amines. Heikkila and Cohen (1974) have shown that T also moderately inhibits DA uptake. Furthermore, decreases in the levels of DOPAC and HVA were observed in the basal ganglia following injections of [^{2}H]-T into the nucleus accumbens (Marien *et al.*, 1987) and following *p*CPA treatment (Fuenmayor and Bermudez, 1985). The effect of *p*CPA, although potentially a result of increased T levels resulting from an increase in the amino acid precursor TRP, may well be due to a direct inhibitory effect on tyrosine hydroxylase activity (Koe and Weissman, 1966). Thus the effects observed in the present experiment reflect many of the findings cited above. The relationship between T and DA is by no means a singular one as T is also known to be a good inhibitor of 5-HT uptake and a strong stimulator of 5-HT release (Horn, 1973; Baker *et al.*, 1977). The increase in the level of 5-HT in the hypothalamus and the decrease in the level of 5-HIAA in the olfactory tubercle appear to indicate a regionally specific T-induced blockade of 5-HT uptake.

CET administration resulted in increased T levels in both brain and liver, thus conforming to the principal action of a prodrug. MAO activity was unaffected by CET treatment. The effects of CET administration on regional amine metabolism varied between the measured amines, DA and 5-HT. One effect of CET administration (with respect to DA metabolism) involved the reduction of acid metabolite (DOPAC and HVA) levels without any effect on DA levels. This action of CET has been attributed to DA uptake blockade (Baker *et al.*, 1987). The second effect was a decrease in 5-HIAA levels accompanied by an increase in 5-HT levels. This latter effect resembles MAO inhibition. Indeed, Rao (1987) had observed

minimal, yet selective MAO-A inhibition *in vitro*, and negligible MAO-A inhibition *in vivo*, therefore suggesting reversible MAO-A inhibition. The effects of CET administration differed from those following T administration, thus diminishing the probability that the actions of CET are mediated solely through an increase in the availability of brain T. It therefore appears that CET acts in a manner which is inconsistent with that of the theoretical prodrug. The increases in whole brain and liver 5-HT levels, without any evidence of CET-induced MAO inhibition, are more difficult to explain and will require further investigation. Preliminary studies rule out the possibility of a contamination of the prodrug with 5-HT.

Although the antidepressants used in this last study were administered acutely, their effects on amine metabolism are very similar to that observed following chronic administration (see Section 4.D). Interactions with T/CET were observed throughout most of the regional HPLC analysis. This was shown to be mainly an interaction between clorgyline and CET. The interactive effect appeared to be a masking of any effect CET had elicited by a clorgyline-induced ceiling effect (i.e. clorgyline, at this dose, induced an increase or decrease in the parent compound or the metabolite, respectively, which apparently masked the lesser changes incurred with CET).

4.E.7 Acute administration of the T prodrug, N-propargyl-tryptamine (PGT): Neurochemical and locomotor effects

Introduction: Preliminary investigations into the potential of PGT as a T prodrug were conducted.

Results: Rats received injections of PGT (0.03, 0.1 and 0.3 mmol kg^{-1} *sc*, 15 min) and the brain tissue was analyzed for T levels and MAO activity as previously described. T levels increased in a dose-dependent manner [$F(3,28)=41.67$] (Table 33). MAO activity was significantly inhibited by PGT [$F(3,27)=83.87$ and $F(3,28)=110.57$ for MAO-A and -B, respectively] although a plateau effect was apparent at the mid-range dose (Table 34). A

PGT (mmol kg^{-1})	Tryptamine ng g^{-1}
Vehicle	n.d.
0.03	15 (2)*
0.1	78 (5)*
0.3	177 (23)*

Table 33: Whole brain tryptamine (T) levels following acute administration of N-propargyltryptamine (PGT) (0.03, 0.1 or 0.3 mmol kg^{-1} sc, 15 min). Mean (ng g^{-1} wet weight tissue) ± (SEM); *: significantly different from vehicle-treated control levels (<1 ng g^{-1}).

	MAO Inhibition (%)	
PGT (mmol kg^{-1})	MAO-A	MAO-B
0.03	20 (3)*	31 (2)*
0.1	52 (2)*	57 (4)*
0.3	53 (4)*	67 (4)*

Table 34: Level of MAO inhibition achieved in brain tissue following acute administration of N-propargyltryptamine (PGT) (0.03, 0.1 or 0.3 mmol kg^{-1} sc, 15 min). Values represent % inhibition relative to vehicle-treated controls. Mean ± (SEM); *: significantly different from control levels.

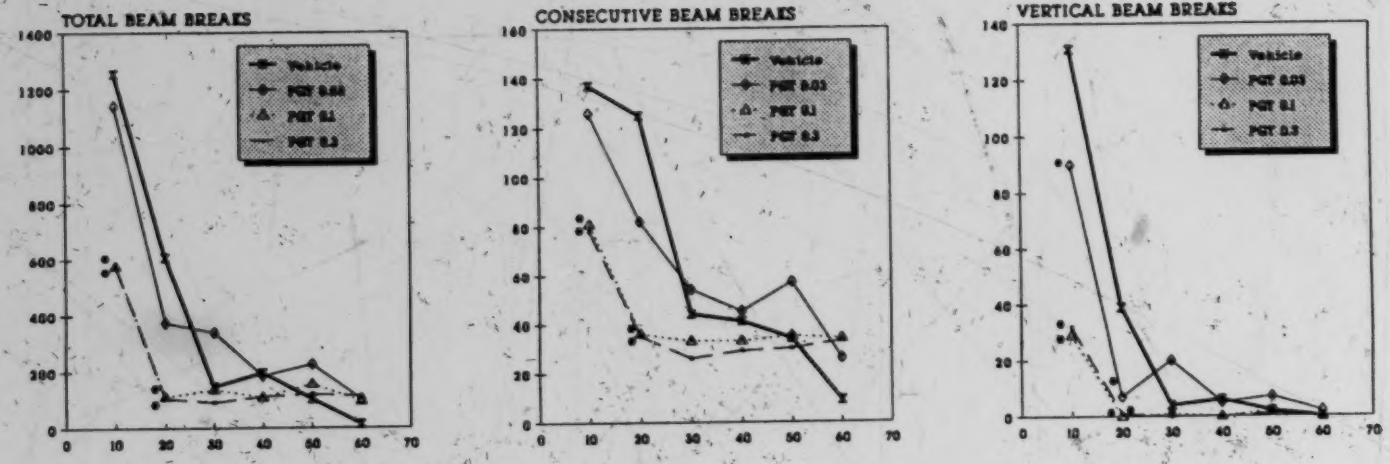


Figure 34: Effect of PGT (0, 0.03, 0.1 and 0.3 mmol kg^{-1} *sc*, 0 min) administration on spontaneous locomotor activity in the rat. *: significantly different from vehicle control.

dose- [F(3,28)=8.05] and time-dependent [F(11,308)=74.85] locomotor (total activity) suppressant effect was observed in rats having received the same drug dose (0 min). An interaction between dose and time was also evident [F(33,308)=4.87; Figure 34]. Similar effects were observed for consecutive activity [dose: F(3,28)=6.77; time: F(11,308)=28.33; dose x time: F(33,308)=3.66; Figure 34] and for vertical activity [dose: F(3,28)=13.92; time: F(11,308)=39.21; dose x time: F(33,308)=4.29; Figure 34].

Discussion: This prodrug acts in a dual fashion in that it results in an elevation in brain T while also having MAO-inhibiting activity. Although both of the prodrugs studied in this series of experiments have been shown to be effective delivery systems for T, their MAO-inhibiting qualities [CET: reversible (Rao, 1987); PGT: at least partially irreversible (present study)] might decrease the effectiveness, as simple prodrugs, of either or both of these compounds. The locomotor suppressant quality of PGT, being very similar to that of CET and the combination of clorgyline and T, might simply be a reflection of increased T availability.

5 GENERAL DISCUSSION

T has been implicated in the etiology of various psychiatric disorders including depression (Dewhurst, 1968a,b). The observation that MAO inhibitors induced elevations in brain T levels (Marsden and Curzon, 1974; Juorio and Durden, 1984; Baker *et al.*, 1985) and that the therapeutically efficacious selective MAO-A inhibitor clorgyline increases brain T levels (Philips and Boulton, 1979) strengthened this hypothesis. Specific binding sites for T were first demonstrated in 1982 (Kellar and Cascio, 1982; Perry *et al.*, 1982). Several groups have since found a decrease in [^{3}H]-T binding site density following chronic MAO inhibitor treament (Cascio and Kellar, 1986; Martin *et al.*, 1987). This is consistent with the general principle of receptor regulation which states that a decrease in binding site number would occur in compensation for the increased availability of the endogenous ligand (Cannon and Rosenblueth, 1949; Creese and Sibley, 1981). In the present study such effects were demonstrated. Chronic administration of the MAO-inhibiting drugs tranylcypromine, phenelzine, clorgyline and (-)-deprenyl resulted in a significant decrease in cortical [^{3}H]-T binding site density. The effect of (-)-deprenyl was unexpected as Martin *et al.* (1987) had demonstrated a lack of effect of this drug on [^{3}H]-T binding; However, this may be explained by examining the route of administration of the compound. Martin *et al.* (1987) administered the drugs in their study via *ip* injection, whereas *sc* injections were used in the present study. This difference in route of administration has been shown by Waldmeier *et al.* (1981) to result in a more potent brain MAO-inhibiting activity following *sc* injection, presumably as a consequence of diminished first-pass metabolism of the compound. Thus, in terms of neurochemical availability, T appears to be a substrate for both forms of MAO. This fact had been suggested earlier following kinetic studies of T metabolism (Neff and Yang, 1974; Sullivan *et al.*, 1986). The chronic administration of tricyclic antidepressant drugs resulted in unaltered [^{3}H]-T binding site density. This is consistent with the recent observation that [^{3}H]-T binding remains unaltered following chronic amitriptyline or trimipramine treatment

(Juorio *et al.*, 1990). Earlier findings which stated that the uptake of T into brain tissue slices and platelets is impeded by 5-HT uptake blockers (Jones and Broadbent, 1982a; Dyck 1984a; Segonzac *et al.*, 1984) seem to contrast with the [^{3}H]-T binding results of the present study. This might be difficult to explain if not for the finding that the uptake of T consists primarily of a passive diffusional process and the active process is almost negligible (Dyck, 1984a,b, 1989). The finding that [^{3}H]-T binding sites do not exist on human platelets (present study), whereas [^{3}H]-imipramine binding sites do (Langer *et al.*, 1983; Wennogle and Meyerson, 1983) raises certain interesting questions. Both of these research groups demonstrated an allosteric interaction between T and 5-HT at the level of the [^{3}H]-imipramine binding site. If there are no platelet [^{3}H]-T binding sites, however, perhaps this suggests a direct action of T at the 5-HT uptake site. This possibility has been forwarded in the context of thermoregulation, vasodilation and modulation of neuronal cell firing rate (see Section 2.C.4). Although this possibility exists, the binding protocol used in the present study to investigate platelet [^{3}H]-T binding required the presence of NaCl in the preincubation and incubation buffers. This might prove to be a serious confounding factor since it has been demonstrated in this present series of experiments, as well as by other researchers (Whittaker, 1969; Boulton and Baker, 1974; Cascio and Kellar, 1983), that the presence of Na^+ ions in the tissue preparation resulted in diminished [^{3}H]-T binding and/or T accumulation. Thus, the lack of [^{3}H]-T binding in the human platelet preparations used in the present study might well be a result of Na^+-induced inhibition of [^{3}H]-T binding. Further studies are therefore necessary to investigate the possibility of platelet [^{3}H]-T binding sites. The inhibition of [^{3}H]-T by monovalent ions (K^+ and Na^+), and specifically Na^+ within its physiological range, could have interesting clinical implications. If T is a neuromodulator, rather than a neurotransmitter (Jones, 1982d), then Na^+ ions might well limit the action of this trace amine under normal homeostatic conditions. However, if electrolyte balance is upset, as sometimes happens in disease conditions, then the modulatory actions of T might be impeded or exaggerated, depending on whether Na^+ concentrations are increased or

decreased, respectively. Given the three examples of T and 5-HT interactions described in the literature review (see Section 2.C.4 on thermoregulation, vasoconstriction and firing rates of neurons), a dysfunction in modulation by T could well affect these physiological activities. It is also interesting to note that this study revealed an age-induced increase in cortical [^{3}H]-T binding site density. Although MAO-A activity levels were found to decrease in the present study, MAO activity was only measured in rest of brain (i.e. brain less cortex). This does not necessarily reflect what occurs in cortical tissue. Indeed, cortical MAO-A activity has been demonstrated to increase with age (Petkov *et al.*, 1987). This would presumably result in decreased levels of cortical T and a compensatory increase in [^{3}H]-T binding site density, in accord with the principles of receptor regulation (Cannon and Rosenblueth, 1949; Creese and Sibley, 1981). The functional relevance of an age-induced increase in [^{3}H]-T binding site density is as yet unknown.

The finding in the present study that the [^{3}H]-T binding site is proteinaceous in nature confirms earlier reports (Brüning and Rommelspacher, 1985, 1989). That this protein binding site is not MAO, as originally proposed by Kienzl *et al.* (1985), was validated by Perry *et al.* (1988). Acute doses of pargyline (50 and 100 mg kg^{-1}) employed in a pilot study in the present series of experiments further substantiates this finding. Neither dose of pargyline altered rat cortical [^{3}H]-T binding, although MAO-A and -B activity was inhibited by $\geq$88% and $\geq$82%, respectively, following both dose treatments. The discovery of specific binding sites for T (see above), as well as the observation that T stimulates phosphoinositol hydrolysis (Osborne and Hogben, 1988), lent credence to the suggestion that T might act as a classical neurotransmitter. Furthermore, the notion that [^{3}H]-T binding sites are functionally significant was supported by their responsiveness to drug treatment. A functional assessment of the [^{3}H]-T binding site was conducted. With this *in vivo* approach, emergent changes in receptor sensitivity are indicated by altered behavioural responses to a selective agonist (T). Altered functional sensitivity of α_2-adrenoceptors has been demonstrated following chronic clorgyline, tranylcypromine and desipramine treatments

(Spyraki and Fibiger, 1980; Cohen *et al.*, 1982; Greenshaw *et al.*, 1988), whereas similar effects on β-adrenoceptor responses were observed following chronic imipramine, desipramine, amitriptyline and mianserin treatments (Przegalinski *et al.*, 1983). The drugs used in the present study were tranylcypromine, clorgyline, imipramine, desipramine and clomipramine. The first two were shown to decrease [^{3}H]-T binding site density, whereas the last three did not. It was hypothesized that T-induced locomotor suppression would be attenuated in the tranylcypromine- and clorgyline-treated rats as these animals would have less [^{3}H]-T binding sites available. The results of this experiment were interesting in that the only treatment to alter T function was clorgyline. In fact, a stimulant effect was observed in these animals, rather than the expected attenuation of the suppressant effect. Zetterstrom *et al.* (1988) and Butcher *et al.* (1990) both have proposed that MAO inhibition increases the synaptic pool of DA available for release. The possibility that T was interacting with an increased availability of endogenous DA (as a result of MAO-A blockade by clorgyline) was overruled by the subsequent observation that T does not potentiate amphetamine-induced hyperactivity. That clorgyline potentially blocked the access of T at the [^{3}H]-T receptor, and thus induced an action of the accessible T at a site other than the [^{3}H]-T binding site is possible, since clorgyline is known to have a high affinity for the [^{3}H]-T binding site (K_i value range; 1.2-2.0 μM: Graham and Langer, 1987; Perry *et al.*, 1988). Another possibility is that clorgyline induced changes in another neurotransmitter system. Clorgyline is known to increase levels of 5-HT, NA and DA (Cohen *et al.*, 1985; O'Regan *et al.*, 1987; present study). T in the nucleus accumbens has been shown to modulate DA-mediated locomotor activity (Marien *et al.*, 1987). In the present series of experiments, both acute and chronic clorgyline treatment resulted in similar increases in DA levels in the striatum, a region associated with the nucleus accumbens in controlling locomotor function. The regionally specific increases in DA cannot explain, however, the dissimilar T-mediated locomotor effects following acute and chronic clorgyline treatment [motor suppression (see below) and motor excitation, respectively]. It is possible that chronically administered

clorgyline mediates an action on DA function other than blockade of catabolism *via* MAO inhibition. The opiates, particularly morphine, are known to differentially affect regional DA metabolism (Kim *et al.*, 1986). Clorgyline displays a high affinity for opiate σ-receptors (Itzhak and Kassim, 1990). In addition, the relatively selective σ-receptor ligand (+)-[^{3}H]-3(3-hydroxyphenyl)-N-1-(propyl)-piperidine (Snyder and Largent, 1989) shares a common piperidine nucleus with the DA uptake site ligand [^{3}H]N-{1-[2-benzo(*b*)thiophenyl]cyclohexyl}piperidine (Vignon *et al.*, 1988). Speculation on a concurrent action of clorgyline at the level of the DA uptake site and the σ-receptor might explain the T-mediated behavioural effects induced by chronic administration of this antidepressant drug. Interestingly, the rank-order of affinities of various MAO inhibitors (clorgyline> (+)-deprenyl> (-)-deprenyl> pargyline) for the σ-receptor is similar to that observed in characterization studies of the [^{3}H]-T binding site, although the affinities are an order of magnitude lower for the latter site (Graham and Langer, 1987: Perry *et al.*, 1988; Itzhak and Kassim, 1990). The earlier observation that neuroleptics block T-induced behaviours (Tedeschi *et al.*, 1959; Squires, 1978) and the recent observation that haloperidol has a high affinity for the σ-receptor (Itzhak and Kassim, 1990) further strengthens the proposed interaction between the DA and opiate systems, as well as an implication of the T system. The effect of chronic clorgyline on T-induced behaviour, however, remains unexplained. The lack of effect in the tranylcypromine-treated animals further suggests that T-induced behaviour is not simply a result of action on an independent T binding site. The lack of concordance between the functional T and apomorphine probes discounts any possibility of an interaction between the T system and DA D_2 presynaptic receptors.

A series of experiments involving acute and chronic (28 d) clorgyline and clomipramine administration were conducted to compare the neurochemical and behavioural effects of these treatments. The effects of acute and chronic clorgyline and clomipramine drug treatments revealed comparable results between the two time-courses of treatment. The MAO inhibitor clorgyline (acute: 1 mg kg^{-1} *sc*, 4 h; chronic: 1 mg kg^{-1} *sc*, 28d Alzet osmotic

minipumps) induced increases in parent amines and a decrease in acid metabolites consistent with previous observations (Cohen *et al.*, 1985; O'Regan *et al.*, 1987). Clomipramine (acute: 10 mg kg^{-1} *sc*, 15 min; chronic: 10 mg kg^{-1} *sc*, 28 d Alzet osmotic minipumps), being a potent 5-HT uptake inhibitor (Randrup and Braestrup, 1977), exerted the majority of its effects on 5-HT metabolism reflected in the decreases in 5-HIAA levels seen throughout most of the brain regions studied. Chronic clomipramine treatment was observed to induce a decrease in the DA metabolites in the frontal cortex and hippocampus. This effect might be attributable to the relatively potent inhibiting effect this tricyclic antidepressant drug exerts on DA uptake (Randrup and Braestrup, 1977). In addition, regional concentrations of clomipramine have been shown to be greatest in the frontal cortex and hippocampus (Kurata *et al.*, 1986). The possibility of this effect being mediated by a metabolite of clomipramine cannot be excluded, however, as comparable effects have been observed with regard to NA metabolism (Flament *et al.*, 1987). These authors found significant levels of the 8-hydroxy and 8-hydroxydesmethyl metabolites of clomipramine in plasma following its administration to human volunteers. Interestingly, levels of DOPAC and 5-HIAA were increased in the hypothalamus following acute injection of clomipramine in the present study, indicating possible enhanced DA and 5-HT release, under these circumstances, in this brain region.

The effects of acute T administration to animals appears to be predominantly excitatory, with a T-induced 5-HT syndrome and increased vocalization as measured parameters (Tedeschi *et al.*, 1959; Foldes and Costa, 1975; Irons *et al.*, 1984; Delini-Stula and Radeke, 1985), although exceptions (i.e. catatonia, rigid posture) have also been reported (Ernst *et al.*, 1961; Schain, 1961). In the present study the behavioural effects of acute T were not evident unless the animals were pretreated with a MAO inhibitor (i.e. clorgyline; 1 mg kg^{-1} *sc*, 4 h), at which time a significant motor suppressant effect was observed (implications of the difference in T-induced behaviours following acute *vs* chronic clorgyline treatments are discussed above). Acute pretreatment with a 5-HT uptake blocker (i.e. clomipramine; 10

mg kg^{-1} *sc*, 15 min) did not result in any overt effect, thus suggesting that, at least behaviourally, T does not appear to elicit its effects directly via the 5-HT system. The absence of any observable increase in brain or liver T levels following clorgyline treatment might simply be attributed to the level of sensitivity of the assay procedure used in this experiment, since previous investigations employing the more sensitive mass spectrometric analysis revealed increases in brain T following MAO inhibitor treatment (Warsh *et al.*, 1977b; Durden and Philips , 1980; Juorio and Durden, 1984). The observation that both clorgyline and (-)-deprenyl induced similar effects on locomotor counts indicates that T is a substrate for both forms of the MAO enzyme. This effect was observed earlier in the present series of experiments when both of these MAO inhibitors induced a decrease in [^{3}H]-T binding site density.

Simultaneous parallel studies were conducted with the potential prodrug for T, N-2-cyanoethyl-T (CET). It was hypothesized that the effects of T and CET on locomotor behaviour would be equivalent if the prodrug was acting simply as a prodrug and thus delivering T to the target site (i.e. brain). Although a potentiation by clorgyline of CET-induced locomotor suppression was observed in the consecutive activity category (0.3 mmol kg^{-1} CET, at 10 min), the predominant observation following clorgyline pretreatment was a lack of potentiation of CET-induced effects. In contrast, clomipramine pretreatment resulted in a potent potentiation of CET-induced locomotor suppression. Subsequent neurochemical analysis revealed increased T levels, both centrally (slight) and peripherally, following CET treatment. In this way CET was fulfilling its prodrug role. Rao (1987) attributes the apparent lack of substantial T levels following CET administration to two possibilities. First, metabolic N-dealkylation might not occur at a rate fast enough to maintain adequate T levels [i.e. perhaps N-dealkylation proceeds at a rate slower than the rapid MAO-mediated deamination of T: Durden and Philips, 1980]. Another possibility is that the rapid rate of clearance of CET from the brain does not allow CET to be metabolized extensively to T in brain.

It was also revealed that CET increases 5-HT levels in whole brain. Potential contamination of the prodrug with 5-HT was discounted. Rao (1987), investigating MAO activity in CET-treated rats, observed slight, yet selective MAO-A inhibition *in vitro*, although negligible effects were observed *ex vivo*. This suggested a potential for reversible MAO-A inhibiting activity. This possibility is perhaps more appropriate since regional analyses of biogenic amines and their acid metabolites revealed changes(in all of the regions studied) in respective levels similar to those observed following MAO inhibitor treatment (i.e. increases in the parent compounds DA and 5-HT and decreases in their respective acid metabolites DOPAC and HVA, and 5-HIAA). Decreases in DOPAC, without concurrent increases in DA levels, following CET treatment has been suggested as indicating a blockade of DA uptake (Baker *et al.*, 1987). The effects of T on amine metabolism were more diverse and appear to be regionally specific. In the frontal cortex T induced an increase in DOPAC levels, whereas decreases in HVA were apparent in the striatum. Baker *et al.* (1987) suggested that this effect is one of predominance of DA release over inhibition of its uptake. Baker and co-workers had previously demonstrated a T-enhanced release of DA from striatal prisms (Baker *et al.*, 1980b; Baker and Yasensky, 1981), although Ennis *et al.* (1981) found that T attenuated the K^+-stimulated release of DA. Interestingly, Baker *et al.* (1980b) also demonstrated a relatively potent inhibitory effect of T on DA uptake. The effects of T on 5-HT metabolism again appeared to be regionally specific with an increase in 5-HT levels in the hypothalamus and a decrease in 5-HIAA levels in the olfactory tubercle. This would appear to indicate a T-induced release of 5-HT in these areas. Similar observations have been made previously (Baker *et al.*, 1977, 1980b; Robinson and Marsden, 1984), although Cox and coworkers have reported an attenuation of K^+-stimulated 5-HT release by T (Cox *et al.*, 1981; Ennis and Cox, 1982). It is obvious that the effects of CET go beyond the simple prodrug effect and that neurochemical activity of the prodrug itself might well explain the differences in T- and CET-induced behavioural effects.

Investigations into the potential of PGT as a T prodrug indicate that, neurochemically, this compound is an effective delivery system for T. The N-propargyl moeity appears to have significant MAO-inhibiting activity, although not as substantial as clorgyline. This is interesting since the levels of brain T following PGT treatment were measurable with the present assay technique, whereas brain T levels following clorgyline treatment were not. This suggests that the PGT-induced increase in brain T levels is primarily a result of delivery, rather than a result of MAO inhibition. Thus, the locomotor suppressant effect observed following PGT administration might well be due to an increase in brain T content, although alterations in the metabolism of other amines susceptible to MAO-mediated catabolism cannot be discounted.

6 CONCLUSIONS

The following conclusions result from the present series of experiments:

1 The [^{3}H]-T binding site is a proteinaceous site which increases with age in the rat and is sensitive to alterations in buffer constituents and storage conditions. Of the buffer concentrations studied, the 50 mM TRIS buffer results in the highest amount of specific [^{3}H]-T binding. Of the cations investigated (i.e. Na^+, K^+, Ca^{++}, Mg^{++}), the monovalent ions are much more potent at inhibiting [^{3}H]-T binding than are the divalent cations. Storage temperature is important when considering the preparation of tissue homogenate as extreme temperatures [i.e. freezing (-80°C) and 37°C] reduce [^{3}H]-T binding significantly, whereas storage of the tissue homogenate at 4°C (12 h) does not alter binding parameters. The observations of ion-dependency and age-related plasticity of the [^{3}H]-T binding site may prove to be important in the understanding of the significance of this functional binding site in the mammalian nervous system.

2 [^{3}H]-T binding is differentially affected by antidepressant drug treatment. A chronic MAO inhibitor regimen induces a decrease in [^{3}H]-T B_{max}, whereas a similar tricyclic antidepressant regimen is without effect. In addition, T appears to be a substrate for both forms of MAO as [^{3}H]-T binding is similarly affected by both non-selective and selective (type-A or -B) MAO inhibitors. The differential effects of tricyclic and MAO inhibitor antidepressant drugs on [^{3}H]-T binding may preclude a general role of T in the mediation of antidepressant drug action. The possible role of T in mediating pharmacotherapeutic effects of MAO inhibitors is, however, supported by the present data.

3 Functionally, exogenous T did not appear to exert its effects directly *via* an action on the [^{3}H]-T binding site since the behavioural effects of T in drug-treated rats did not correlate with the changes in [^{3}H]-T binding site density following equivalent drug regimens. Neither did T exert its effects *via* an action on, or through cooperativity with,

the presynaptic D_2 DA receptor. T did not appear to exert its behavioural effects *via* an action on endogenous DA.

4 The cyanoethyl analogue of T, CET, may act as a prodrug of T as its administration results in an increased availability of T in the brain. However, contrasting effects of clorgyline and clomipramine pretreatments on CET- and T-induced locomotor activity in the rat suggest an action of CET other than the simple delivery of T to its site of action.

5 The potentiation of T-induced locomotor suppression by both clorgyline and (-)-deprenyl substantiate the conclusion (see point 2 above) that T is a mixed substrate for MAO.

6 Clorgyline and clomipramine differentially affect regional brain biogenic amine concentrations. Their respective effects are evident whether the drug is administered acutely or chronically. The specificity of clomipramine as a 5-HT uptake inhibitor appears to vary regionally as clomipramine (administered chronically) also affects DA metabolism in the frontal cortex and hippocampus of the rat.

7 The observed stimulant effect observed following administration of T to rats treated chronically with clorgyline suggests an action of this latter compound other than the simple inhibition of MAO since rats treated with another MAO inhibitor (tranylcypromine) did not display similar locomotor stimulant behaviour.

8 The propargyl analogue of T, PGT, appears to have great potential as a prodrug of T although its capacity for MAO inhibition may be responsible for certain of its effects, notably its locomotor suppressant effects.

7 FUTURE STUDIES

Certain features of this thesis work necessitate further investigation:

1. The similar effects of CLG and DEP on [^{3}H]-T binding site density, combined with their selectivity in terms of MAO inhibition, indicate that only a fraction of MAO needs to be inhibited for [^{3}H]-T binding site down-regulation. It is also a possibility that these selective MAO-inhibitors block a subtype of MAO or a functionally similar oxidizing enzyme for which T has a higher affinity *in vivo*. The degree of MAO inhibition required for T accumulation and for down-regulation of the [^{3}H]-T binding site, as well as the specificity of T as a MAO substrate therefore should be investigated. The effects of MAO inhibitors on [^{3}H]-T binding site density should be investigated in different brain regions to assess the generality of the effects in cortex.

   In addition, if the gene for the site is cloned, it would be of interest to investigate the possibility of gene regulation of the [^{3}H]-T binding site. The actual function of the [^{3}H]-T binding site could then be investigated using transfection techniques.
2. The stimulant effect observed following the T challenge in CLG-treated animals deserves definite pursuit. The possibility of an interaction between T and a receptor with a high affinity for CLG, this receptor perhaps being the sigma receptor, should be investigated. Sigma receptor agonists and antagonists could be used in behavioural tests and using radioligand binding procedures to determine the extent of interaction between T and/or CLG and this receptor.
3. The large increases in brain and liver 5-HT levels following acute CET administration indicate a potential for this compound as a useful tool for manipulating 5-HT activity. Thus, further studies of the metabolism of CET will be of interest.
4. The neurochemical and behavioural effects of PGT should also be pursued. The large accumulation of brain T following administration of this compound, as well as the limited degree of MAO inhibition, indicate a potential for PGT as a T prodrug.

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